



EXHIBIT A

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DEC 31 2002

TECHNOLOGY CENTER 2300

Lab notebook 1 PT 1

TITLE

PROJECT NO.

BOOK NO.

11

DUPLICATE

Laser diode now in use:

NVG Inc 640nm

5mW laser

ordered from Digix 70-90 milliamps

NVG part no. M640.5.I

Also, think about using super-bright LED !!

Now trying pyrex tube flow cell (instead of the soft glass previously used) to better match index of refraction w epoxy.

SIGNATURE

Richard W. Shorehill

DATE

7/5/99

WITNESS

5/3/99

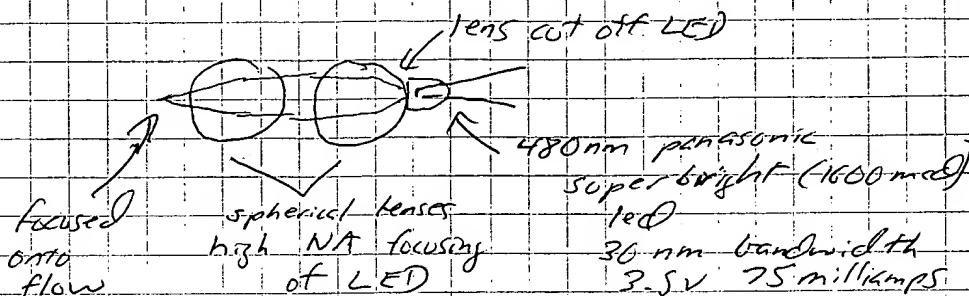


TITLE

PROJECT NO.
BOOK NO.

12

Fluor Fluorescein system 480nm LED

Illumination with blue LED

Fluorescein at ~~50~~ 520nm
use Edscorp 520nm interference filter
for imaging

ordered 8mm BK-7 spheres from Edscorp

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SIGNATURE

DISCLOSED TO AND UNDERSTOOD BY

Richard W. Shontell

DATE

7/5/99

WITNESS

DATE

5/10/99

DATE

TITLE

PROJECT NO.

12

BOOK NO.

DUPLICAT.



FLUORESCENCE SET: XF25

FOR DYES: RH 414, RD1, Fura Red™/ Fluo-3, FM1-43, CR6G, Calcein BODIPY®-Ceramide, Nile Red, fluo-3, FITC, BODIPY®-FL, BCECF, DIO, YOYO™-1, Calcium Green™, Y/G FluoSpheres®, TOTO™-1, rhodamine 123, ethidium-homodimer

EXCITATION: 485DF22 (FULL LINE)

DICHROIC: 505DRLPO2 AT 45° AOI (CHAIN LINE)

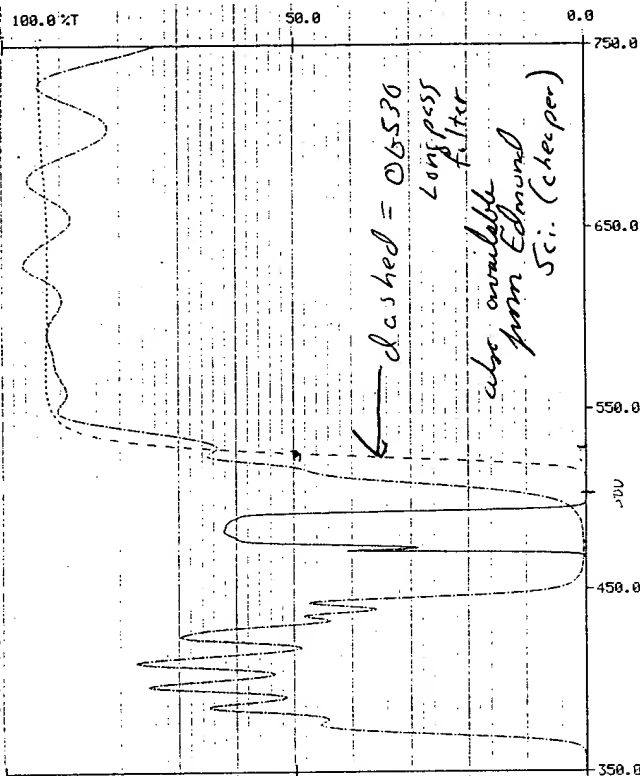
EMISSION: OG530 (BROKEN LINE)

SPECTRAL CONTROL:

EXCITATION: 0.8 CWL TO FAR IR X-AXIS: 350-750 nm X-SCALE: 10 nm/div.

EMISSION: X-RAY TO 1.2 x CWL Y-AXIS: 0-100%T

Arrows on excitation and emission filters point in the direction of light path
Not recommended for use with the following light source(s): Hg



OMEGA OPTICAL, INC. • TEL: (802) 254-2690 • FAX: (802) 254 3937

SIGNATURE

DISCLOSED TO AND UNDERSTOOD BY

DATE

WITNESS

DATE

Richard W. Shortell

7/5/99

6/9/99

Long-pass filter
fluorescence

TITLE

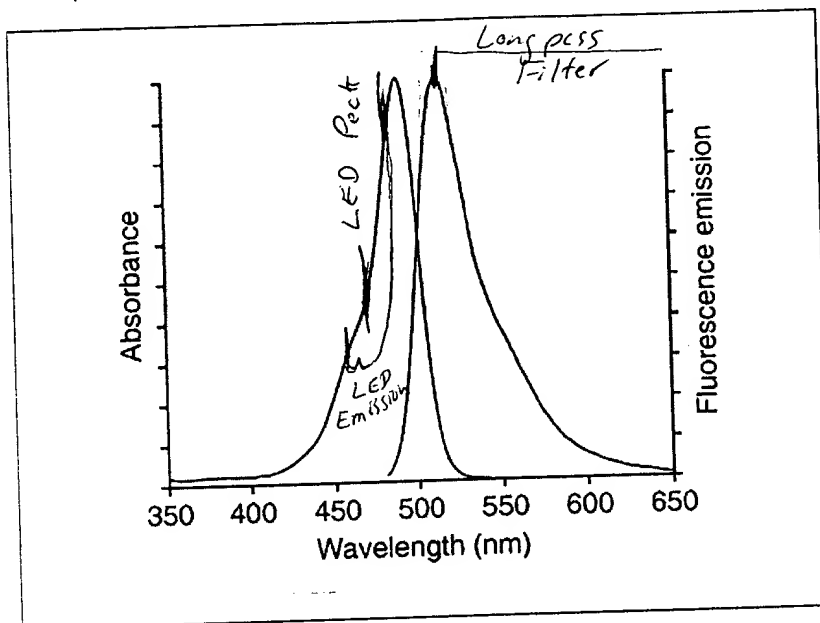
PROJECT NO.

BOOK NO.

13

Spectra

Absorption and fluorescence emission spectra of fluorescein (F-1300) in pH 9.0 buffer.



From Molecular Probes Web Site

LED emits at 470 nm! (nominal)
with $\Delta\lambda = 30\text{ nm}$

SIGNATURE

DISCLOSED TO AND UNDERSTOOD BY

Richard V. Shorrock

DATE

7/5/99

WITNESS

DATE

6/9/99

DATE

TITLE

PROJECT NO.

BOOK NO.

7/8/99

1:

To run bioimage in EDL

cd, 'cildan' get into "home" directory
compile bioimage
@test

We measured Argon laser power:

0.5 mw minimum pot. setting

8 9 mw max

Using power meter calibrated at 628 nm.

These estimates are not corrected.

with new coherent power meter from Edmund

we get 20 mw max.

2 mw min.

Tested blue green Nichia LED with
490-495 nm peak (10 ma)

1 mw power at 100 ma current

at 200 ma device is destroyed

We might run at 100 ma.

SIGNATURE

[Signature]

DISCLOSED TO AND UNDERSTOOD BY

Michael Hunt

DATE

4/2/02

WITNESS

DATE

7/8/99

DATE

TITLE

To Do/Improvements

PROJECT NO.

BOOK NO.

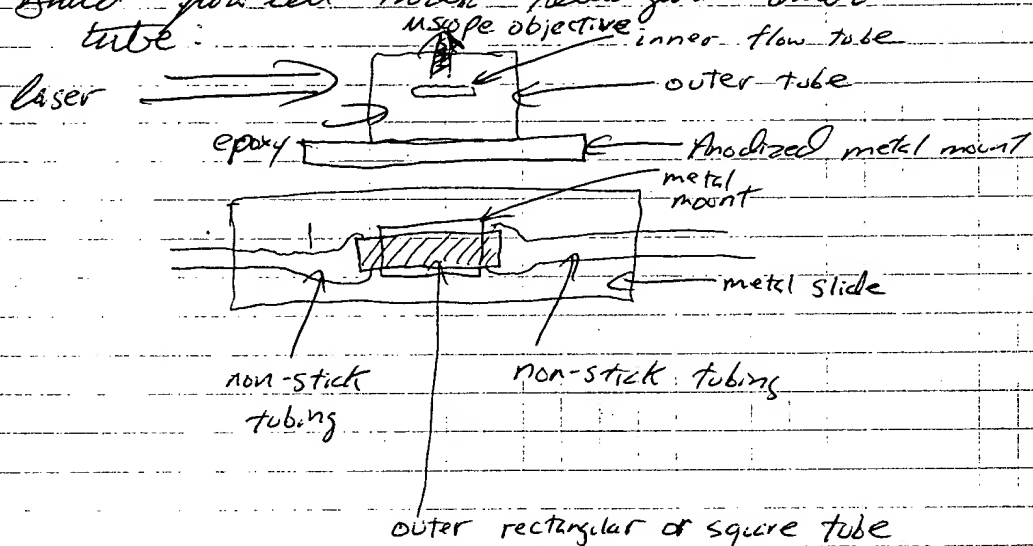
236

DUPLICATE

Note: 1. to prevent bacteria clumping after centrifuge step — think about using a "anti-clumping" buffer.

2. Build mechanical stage for microscope w easy mounting and adjustment...

3. Build flow cell with rectangular outer tube:



index match glass and epoxy

4. Build LED collimation/focusing module.

5. Perform statistical tests on bead photometry w BIOCOUNT (improved).

6. Calibrate CCD gain + dark + readout noise
Use slope of variance/exposure curve to calibrate gain

7. Find dark noise in TDI mode along columns and differentiate from imaging dark noise.

SIGNATURE

DISCLOSED TO AND UNDERSTOOD BY

Michael Hund

DATE 9/11/00

WITNESS

10/1/98



EXHIBIT B

RECEIVED

DEC 31 2002

TECHNOLOGY CENTER 2800



September 30, 1999

J. Winslow Young
Registered Patent Agent
P.O. Box 1088
Centerville, UT 84014-5088

Dear Winslow,

Here is an overview of my invention concerning the use of LEDs for flow cytometry in general and CCD/TDI flow cytometry in particular. Please retain a copy of this for your files.

Use of LEDs in CCD/TDI flow cytometer.

The use of LEDs is practical in flow cytometry, especially for some applications, and has the following advantages:

1. LEDs are typically monochromatic and some can currently output radiant power in the 1 mW to 2 mW range. This power range makes them suitable for some immunofluorescent flow cytometry.
2. LEDs are typically very inexpensive and can be purchased for ~1 dollar, making them extremely attractive relative to lasers or incandescent sources.
3. Their low cost and small size makes it easy to replace an LED of a given wavelength with one of another wavelength, in modular fashion. Since a variety of manufacturers make LEDs at wavelengths that nearly cover the entire visible spectrum, it is nearly possible to match any given visible wavelength fluorescent tag to a superbright LED, allowing the fabrication of a general purpose flow cytometer with a large number of inexpensive LED modules.
4. Simple optics (lenses) can be used to focus the LED beam to a smaller beam size permitting a high-intensity illumination spot.

LEDs haven't been previously used for flow cytometry for the following reasons:

1. Many flow cytometers have higher powered lasers (20 to 100 mW) as illumination sources as they are more forgiving of inefficient optical design.
2. It is common for applications requiring precision photometric measurements of particles in a flow cytometer to use the inner part of the Gaussian beam (say, ~5 to 10% of the power) which has a relatively constant or "flat" intensity to illuminate the core flow. This insures that a particle, no matter where it is in the core flow, will receive almost precisely the same illumination. In systems where constant illumination is not critical (e.g. where one simply wants to detect bacteria and not perform precision photometry) LED illumination becomes practical.

We (and others) have shown that flow cytometry can be performed with a beam power of ~2 mW. We have measured the output power of several superbright LEDs and found them to be ~1 mW of radiant power. The trend towards brighter and more efficient LEDs will only improve the performance and cost of LEDs in the future.

TECHNOLOGY CENTER 2000

DEC 31 2002

RECEIVED

Sincerely,

Paul E. Johnson
President, SoftRay Inc.
519 South 5th St.
Laramie WY 82070

P.S. I would also add that the increasing number of wavelengths, the increasing power, and the recent advent of blue LEDs, makes them particularly suitable just now.



EXHIBIT C

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DEC 31 2002

TECHNOLOGY CENTER 2800



SoftRay, Inc.

Confidential

RECEIVED
DEC 31 2002
TECHNICAL CENTER 2800

Oct 12, 1999
September 30, 1999

J. Winslow Young
Registered Patent Agent
P.O. Box 1088
Centerville, UT 84014-5088

Dear Winslow,

Here is a drawing concerning the use of an LED illumination source in flow cytometry in general and CCD/TDI flow cytometry in particular. Please retain a copy of this for your files.

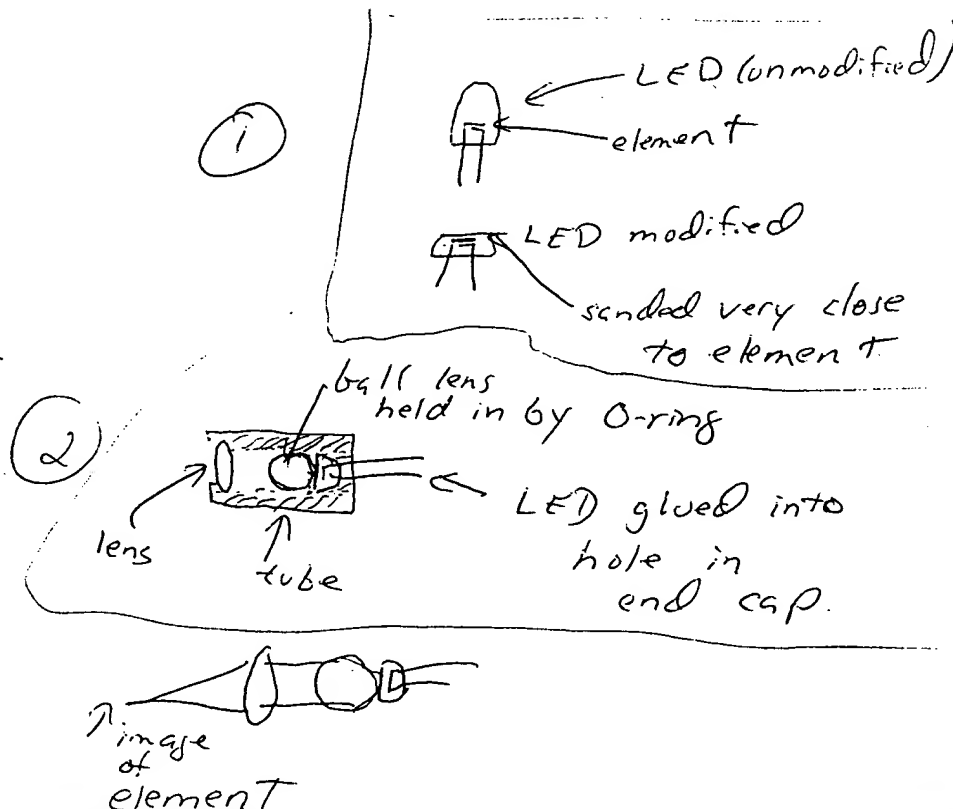
Also note: I sent a "non-disclosure packet" to B&D, which arrived two days before my visit. This included the letter that I previously faxed to you regarding LEDs and flow cytometry. When I arrived, the Director of New Technology, Bob Hoffman, informed me that they hadn't read the document on LEDs (referred to in a cover letter), and didn't want to, because it interferes with their intellectual property. Interesting. They had me remove this letter from the opened express mail packet in their possession. Neither party signed or had witnessed any of this.

Use of LEDs in Flow Cytometry

In the device below, there are three components, an off-the-shelf LED, a collimating lens, and a focusing lens. I have drawn a ball lens as the collimating lens because it has a fast $f/\#$ and can efficiently collect light from the LED. It is commonly used to collect and collimate light from fiber optics. It is most efficient if it makes contact with the LED. The domed surface of an LED acts as a lens, making the LED more directional. I remove this by sawing it off and polishing the end flat with sandpaper or emery paper. The final component is a focusing lens, the size and focal length can be chosen to control the size of the module and the magnification of the final spot. (The magnification is simply the ratio of the focal lengths of the two lenses: $f.l.(focusing \text{ lens})/f.l.(ball \text{ lens})$).

My best,

Paul Johnson
SoftRay Inc.





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DEC 31 2002
TECHNOLOGY CENTER 2000

EXHIBIT D



Purchase Order 040400A

SoftRay Inc.

519 South 5th Street
Laramie, WY 82070
Telephone: (307)-745-3743
Fax: (307)-745-3743

To: Nichia
Date: April 4, 2000
Attention: Michael Stewart

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DEC 31 2001
TECHNICAL SERVICES

ITEM	Description	Number of Units	Unit Price	Price
I	NSPG300A – F Rank (510-520 nm)	10	\$6 Ea.	\$60
Shipping (est.)	Fedex C.O.D.			\$14.50
TOTAL				\$64.50

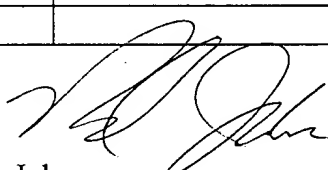

Paul E. Johnson
President, SoftRay, Inc.

EXHIBIT E

Status: U
Date: Fri, 01 Nov 2002 15:11:57 -0700
From: "Paul E. Johnson" <PJohnson@uwyo.edu>
Subject: FW: Respons to NSF 4/28/00
To: bales@mbj-law.com
Thread-Topic: Respons to NSF 4/28/00
Thread-Index: Ab+xaH4luQ1p0h1REdSErQCqAN2PpYWhE9kQAABROwA=
X-OriginalArrivalTime: 01 Nov 2002 22:12:00.0199 (UTC)
FILETIME=[B31F1570:01C281F3]

-----Original Message-----

From: Paul E. Johnson
Sent: Friday, November 01, 2002 3:03 PM
To: Paul E. Johnson
Subject: FW: Respons to NSF 4/28/00

-----Original Message-----

From: Richard W. Shorthill [<mailto:shorth@eng.utah.edu>]
Sent: Friday, April 28, 2000 5:20 PM
To: Paul E. Johnson
Subject: Respons to NSF 4/28/00

Paul:

4/28/00, 5:20PM
Some items need to be submitted to NSF "Division of Contracts, Policy
and
Oversight
Cost Analysis and Audit Resolution Branch" that we discussed also the
last item
Indirect Costs: . (See sample indirect cost proposal found at Appendix 6
of the Guide.). Call me with questions.

Dick

Richard W. Shorthill, Ph. D.
University of Utah
Mechanical Engineering
50 S. Campus Drive
Salt Lake City, UT 84124-9208
Office E-mail: shorth@eng.utah.edu
Home E-mail: srw4@uswest.net
FAX: 801-585-9826
Phone: 801-581-8623

Content-type: application/applefile; name=bruce#5.doc
Content-disposition: attachment; filename=bruce#5.doc
Content-description: bruce#5.doc

bruce#5.doc

HAMILTON: "Administrative and Financial Information for Potential SBIR/STTR Phase II Awards" answers to the questions for "5" below

5. Provide documentation as detailed below to support the proposed costs in the following categories. (In the event, you have already provided any of the requested documentation; there is no need to resubmit it.)

Senior Personnel: (1) Provide current payroll register for each employee.

(1) Payroll register for SoftRay, Incorporated: Dr. E. Johnson, Ph. D., President [Rate: \$34.61/hr]. Dr. Richard W. Shorthill, Ph. D., Senior Scientist and Principal Investigator [Rate: \$23.08/hr].

(2) For each named individual who is not presently employed by the organization, provide an employment agreement stating the rate of payment and also a statement setting forth the organization's considerations in determining the rate of pay offered. (Provide salary survey or other documentation to support proposed rate.)

(3) For positions where specific individuals have not been named, provide an explanation of how the rates were determined and any related documentation.

(3) Employees to be hired by SoftRay, Incorporated after award received: Biologist [Rate \$13.46/hr] based on undergraduate typical rates for a Biologist/ microbiologist in Laramie, Wyoming.

Graduate student [Rate \$15.00/hr] part time employee, SoftRay, Inc. typical rate for starting part time help.

Other Personnel: For all "other personnel" proposed at an annualized salary in excess of \$40,000, provide cost data as described for senior personnel above.

Fringe Benefits: Provide the categories of costs (FICA, health benefits, etc.) included and the related amounts. Provide calculation of the actual fringe rate for previously completed fiscal year (total fringe costs / total labor costs).

Fringe Benefits: FICA 7.65 %; Unemployment 0.5 %; Health insurance, dental care, life insurance, eye glasses, other reasonable and necessary medical procedures; receipts required; The total for all these benefits may not exceed 28% of wages.

Permanent Equipment: (Defined as nonexpendable, tangible personal property, having a useful life of more than one year and an acquisition cost of \$5,000 or more per unit.) Provide cost data from three sources which can be in the form of written quotations and/or copies of pricing information contained in catalogues, trade journals, etc. In situations where equipment can only be provided by one specific source provide a sole source justification and a written quote from this source.

No single item \$5000 or more dollars.

Travel: Provide an itemization of travel which should include the destination, purpose of travel, number of days in travel status, and the estimated costs for items included in the amount (airfare, cab fare, car rental, per diem rates, hotel and other incidentals.)

Travel

E. TRAVEL		1. DOMESTIC	10425
		2. FOREIGN	
name	Purpose	Location #trips \$ ea total	
1 RWS	Project meetings, Soft Ray,Laramie 3 days	SLC/LAR 11 475 5225	
2 PEJ	Technical meeting on Cytometry 4 days	LAR/BOS 1 1400 1400	
3 RWS	Consultant's lab, Bozeman, MT 3 days	LAR/Bozeman 1 900 900	
4 PEJ	Project meetings, Salt Lake City,UT 3 days	LAR/SLC 2 475 950	
5 PEJ	Support meeting w/Becton Dickenson 3 days	LAR/SFO 1 1000 1000	
6 PEJ	Consultant's lab, Bozeman, MT 3 days	LAR/Bozeman 1 950 950	

E. TRAVEL		1. DOMESTIC	\$ 11,150
		2. FOREIGN	
name	Purpose	Location #trips \$ ea total	
1 RWS	Project meetings, Soft Ray, Laramie 3 days	SLC/LAR 10 475 4750	
2 PEJ	Technical meeting on Cytometry 5 days	LAR/SFO 1 1400 1400	
3 RWS	Consultant's lab, Bozeman, MT 3 days	SLC/Bozeman 1 900 900	
4 PEJ	Present Paper on Cytometry 4days	LAR/BOS 1 1200 1200	
5 PEJ	Project meetings, Salt Lake City,UT 3 days	LAR/SLC 2 475 950	
6 PEJ	Support meeting w/Becton Dickinson 3 days	LAR/SFO 1 1000 1000	
7 PEJ	Consultant's lab, Bozeman, MT 3 days	LAR/Bozeman 1 950 950	

Airfare- coach: LAR/Bozeman, LAR/SFO, LAR/BOS, Private Vehicle-SLC/LAR, LAR/SLC, SLC/BOZ, BOZ/SLC, LAR/SLC. Lodging-actual, per diem- \$35/day. Mileage- \$0.31/mile. Cab/Bus Fare-actual. Receipt required for items \$10 or over.

Materials and Supplies: Provide an itemized listing of materials and supplies. Provide quotations or other appropriate documentation to support any single material and supply item with an extended amount in excess of \$5,000.

Materials:

1. MATERIALS AND SUPPLIES 1 st YEAR	22200
1.1 Microscope fluorescence conversion modules 5000	

1.2 Improved TDI/CCD camera	2500	
1.3 Optics:collimators, focusing, reimaging, mirrors	2800	
1.4 Computer	2000	
1.5 Pump: Liquid	400	
1.6 Laser: LED, gas, dye	2000	
1.7 Chemical/biological: labeled antibodies, labeled beads	3500	
1.8 Filters: interference, \$1000 notch m\$1000	2000	
1.9 IDL: Programming Interactive data language	1500	
1.10 Bacteria: simulants	500	
1. MATERIALS AND SUPPLIES 2nd Year		\$ 4,840
1.1 Chemical/biological: markers, dyes, beads	1200	
1.2 Optics: filters(interference, notch), mirrors,	1000	
1.3 Bacteria: simulants	640	
1.4 Laser	2000	

Publication Costs/Documentation/Dissemination: Provide an explanation of items included in this amount and the per item cost (e.g., the estimated number of pages and the per page cost.)

No cost

Consultant Services: For each consultant, provide an agreement which includes the following information: (1) the services to be provided; (2) the period of performance and the consultant's availability; (3) the qualifications of the consultant to perform the work; and (4) the rate of pay (not to exceed \$453 per day). (Consultants should be chosen using a competitive selection process; otherwise, sole source justifications should be maintained.)

3. CONSULTANT SERVICES 1st YEAR				\$7,950
3.1 Douglas A. Christensen, Ph. D. (EE/Bio. Elec. Engr)	16hrs	\$75/hr	1200	
specific work in Bioengineering, Laser technology				
3.2 James J. Smith, Ph. D. (Microbiologist)	66hrs	\$75/hr	4950	

Experimental Design and review
 3.3 Warrie Means, Ph. D. (Meat Scientist) 24hrs \$75/hr 1800
 ", testing meat samples

3. CONSULTANT SERVICES 2 nd YEAR				\$7,950
3.1	Douglas A. Christensen, Ph. D. (EE/Bio. Elec. Engr)	12hrs	\$75/hr	900
3.2	James J. Smith, Ph. D. (Microbiologist)	66hrs	\$75/hr	4950
3.3	Warrie Means, Ph. D. (Meat Scientist)	28hrs	\$75/hr	2100

Each consultant will work not more than 6 hours in any day (\$450) which does not exceed \$453. The original proposal includes their letter of commitment and a resume which demonstrates their qualifications as a consult on this proposal.

Subawards: For each subaward agreement provide (1) the services to be provided, the names of individuals expected to perform the work, and the expected level of effort for each individual; (2) cost information broken out by cost category (salaries, fringe, travel, Subaward to the University of Wyoming

Personnel:

A. SENIOR PERSONAL: PI/PD 1 st YEAR			Period of performance	Funds
1	Dr. Paul E. Johnson PD Direct the subcontract activities		3 months of activity over 1 year	0
2	(To be hired) Engineer Provide mechanical and electronic design for the Cytometer, controls, and tests of the cytometer system		9 months of activity over 1 year	\$21,016

B. OTHER PERSONNEL 1 st YEAR				
3.	GRADUATE STUDENT Assist the Engineer particularly with the testing of system	600 hours		\$9,000
4.	UNDERGRADUATE STUDENT Carry out work assigned and work with the engineer and graduate student	500 hours		\$5,000

G. OTHER DIRECT COSTS 1 st YEAR				
1. MATERIALS AND SUPPLIES				\$7,000
1.1	Mech. & Optical Shop (design construction of cytometer, optics and controls)	112 hrs	\$5,600	
1.2	Graduate student tuition (requirement of the University of Wyoming)		\$1,400	

A. SENIOR PERSONAL: PI/PD 2 nd YEAR			Period of performance	Funds
1	Paul E. Johnson PD		3 months of activity over 1	0

		year		
2	(To be hired) Provide mechanical and electronic design for the Cytometer, controls, and tests of the cytometer system	Engineer	12 months	\$28,020

B 4.	UNDERGRADUATE STUDENT 2 nd YEAR Carry out work assigned and work with the engineer and graduate student	500 hours	\$5,000
------	---	-----------	---------

G . OTHER DIRECT COSTS		
1.1 Mech .& Optical Shop (design construction of cytometer, optics and controls)	112 hrs	\$5,600

For Year 1 and Year 2, **Fringe Benefits: FICA 7.65%; Unemployment 0.5%; Health insurance, dental care, life insurance, eye glasses, other reasonable and necessary medical procedures; receipts required; The total for all these benefits may not exceed 28% of wages.**

The total budgets for the subaward are shown in the first and second year budgets in the original proposal.

Indirect Costs: Provide a current indirect cost rate agreement negotiated by a Federal Agency. In the absence of a current negotiated rate agreement, provide an indirect cost proposal and the related financial statements (income statement and balance sheet) for the two most recently completed annual accounting fiscal periods. If an organization has not had financial activity for these periods, indirect cost proposals should be based on what activities the organization expects during the proposed award period. (See sample indirect cost proposal found at Appendix 6 of the Guide.)

In order to avoid delays in the review and processing of your proposal, please send the requested information within 15 days of the above date to:

To: National Science Foundation
Division of Contracts, Policy and Oversight
Cost Analysis and Audit Resolution Branch
4201 Wilson Boulevard, Room 475
Arlington, VA 22230

Cc: to Bruce Hamilton

Questions related to Items 1 and 2 should be directed to the Division of Grants and Agreements (Attn: Andrea Kline) at 703-306-1212. Questions related to Items 3 through 5 should be directed to the Cost Analysis and Audit Resolution Branch (Attn: Patricia Farrell) at 703-306-1244.

EXHIBIT F

Status: U
Date: Fri, 01 Nov 2002 15:11:49 -0700
From: "Paul E. Johnson" <PJohnson@uwyo.edu>
Subject: email correspondence on LEDs
To: bales@mbj-law.com
Thread-Topic: Bruce #5 Friday 5/5/00
Thread-Index: Ab+2q0sEhBG7sSKYEdSErQCqAN2PpYWWjaFgAABeeuA=
X-OriginalArrivalTime: 01 Nov 2002 22:11:49.0746 (UTC)
FILETIME=[ACE41520:01C281F3]

Jennifer,

This is the first of 4 emails that I sent re: LEDs.

Paul

-----Original Message-----

From: Paul E. Johnson
Sent: Friday, November 01, 2002 3:01 PM
To: Paul E. Johnson
Subject: FW: Bruce #5 Friday 5/5/00

-----Original Message-----

From: Richard W. Shorthill [<mailto:shorth@eng.utah.edu>]
Sent: Friday, May 05, 2000 9:58 AM
To: Paul E. Johnson
Subject: Bruce #5 Friday 5/5/00

Paul:

The #5 items response to Bruce Hamiltons request. Call me at home over the weekend if you have questions.

Dick

Richard W. Shorthill, Ph. D.
University of Utah
Mechanical Engineering
50 S. Campus Drive
Salt Lake City, UT 84124-9208
Office E-mail: shorth@eng.utah.edu
Home E-mail: srw4@uswest.net
FAX: 801-585-9826
Phone: 801-581-8623

Content-type: application/applefile; name=CorNSF5.doc
Content-disposition: attachment; filename=CorNSF5.doc
Content-description: CorNSF5.doc

CorNSF5.doc

Response to BRUCE HAMILTON: "Administrative and Financial Information for Potential SBIR/STTR Phase II Awards" answers to the questions for "5" below

5. **Provide documentation** as detailed below to support the proposed costs in the following categories. (In the event, you have already provided any of the requested documentation; there is no need to resubmit it.)

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(1) Payroll register for SoftRay, Incorporated: Dr. E. Johnson, Ph. D., President [Rate: \$34.61/hr]. Dr. Richard W. Shorthill, Ph. D., Senior Scientist and Principal Investigator [Rate: \$23.08/hr].

(2) For each named individual who is not presently employed by the organization, provide an employment agreement stating the rate of payment and also a statement setting forth the organization's considerations in determining the rate of pay offered. (Provide salary survey or other documentation to support proposed rate.)

(3) For positions where specific individuals have not been named, provide an explanation of how the rates were determined and any related documentation.

(3) Employees to be hired by SoftRay, Incorporated after award received: Biologist [Rate \$13.46/hr] based on undergraduate typical rates for a Biologist/ microbiologist in Laramie, Wyoming.

Graduate student [Rate \$12.00/hr] part time employee, SoftRay, Inc. typical rate for starting part time help.

Other Personnel: For all "other personnel" proposed at an annualized salary in excess of \$40,000, provide cost data as described for senior personnel above.

Fringe Benefits: Provide the categories of costs (FICA, health benefits, etc.) included and the related amounts. Provide calculation of the actual fringe rate for previously completed fiscal year (total fringe costs / total labor costs).

Fringe Benefits: FICA 7.65%; Unemployment 1.5%; WORKMANS COMP., Health insurance, dental care, life insurance, eye glasses, other reasonable and necessary medical procedures; receipts required; The total for all these benefits may not exceed 28 % of wages.

Permanent Equipment: (Defined as nonexpendable, tangible personal property, having a useful life of more than one year and an acquisition cost of \$5,000 or more per unit.) Provide cost data from three sources which can be in the form of written quotations and/or copies of pricing information contained in catalogues, trade journals, etc. In situations where equipment can only be provided by one specific source provide a sole source justification and a written quote from this source.

No single item \$5000 or more dollars.

Travel: Provide an itemization of travel which should include the destination, purpose of travel, number of days in travel status, and the estimated costs for items included in the amount (airfare, cab fare, car rental, per diem rates, hotel and other incidentals.)

Travel

E. TRAVEL		1. DOMESTIC	10425		
		2. FOREIGN			
name	Purpose	Location	#trips	\$ ea	total
1 RWS	Project meetings, Soft Ray, Laramie 3 days. Discuss progress & plans.	SLC/LAR	11	475	5225
2 PEJ	Technical meeting on Cytometry 4 days. Attend technical papers.	LAR/BOS	1	1400	1400
3 RWS	Consultant's lab, Bozeman, MT 3 days. Review microbiology tests.	LAR/Bozeman	1	900	900
4 PEJ	Project meetings, Salt Lake City, UT 3 days. Discuss progress & plans.	LAR/SLC	2	475	950
5 PEJ	Support meeting w/Becton Dickenson 3 days. Review commercialization.	LAR/SFO	1	1000	1000
6 PEJ	Consultant's lab, Bozeman, MT 3 days. Review microbiology tests.	LAR/Bozeman	1	950	950

E. TRAVEL		1. DOMESTIC	\$ 11,150		
		2. FOREIGN			
name	Purpose	Location	#trips	\$ ea	total
1 RWS	Project meetings, Soft Ray, Laramie 3 days. Discuss progress & plans	SLC/LAR	10	475	4750
2 PEJ	Technical meeting on Cytometry 5 days. Attend technical papers.	LAR/SFO	1	1400	1400
3 RWS	Consultant's lab, Bozeman, MT 3 days. Review microbiology tests	SLC/Bozema n	1	900	900
4 PEJ	Present Paper on Cytometry 4days. Present research results.	LAR/BOS	1	1200	1200
5 PEJ	Project meetings, Salt Lake City, UT 3 days. Discuss progress & plans.	LAR/SLC	2	475	950
6 PEJ	Support meeting w/Becton Dickinson 3 days. . Review commercialization.	LAR/SFO	1	1000	1000
7 PEJ	Consultant's lab, Bozeman, MT 3 days. Review microbiology tests.	LAR/Bozema n	1	950	950

Airfare- coach: LAR/Bozeman, LAR/SFO, LAR/BOS, Private Vehicle-SLC/LAR, LAR/SLC, SLC/BOZ, BOZ/SLC, LAR/SLC. Lodging-actual, per diem- \$35/day. Mileage- \$0.31/mile. Cab/Bus Fare-actual. Receipt required for items \$10 or over.

Materials and Supplies: Provide an itemized listing of materials and supplies. Provide quotations or other appropriate documentation to support any single material and supply item with an extended amount in excess of \$5,000.

Materials:

1. MATERIALS AND SUPPLIES 1st YEAR		22200
1.1 Microscope fluorescence conversion modules Optical components \$3,000. Power module \$1,000, mounting attachments \$1,000.	5000	
1.2 Improved TDI/CCD camera	2500	
1.3 Optics: collimators, focusing, reimaging, mirrors. 2ea optical collimators, 4ea focusing lenses, 4ea reimaging lenses, 4ea first surface mirrors	2800	
1.4 Computer	2000	
1.5 Pump: SERINGE PUMP	400	
1.6 Laser: LED, gas, dye. 8ea LEDs, 1ea gas laser, 1ea dye laser.	2000	
1.7 Chemical/biological: Labeled antibodies \$2,000, labeled beads \$1,500.	3500	
1.8 Filters: Interference filters, \$1,000 notch filters \$1,000	2000	
1.9 IDL: Programming. Interactive data language program.	1500	
1.10 Bacteria: simulants	500	

1. MATERIALS AND SUPPLIES 2nd Year		\$ 4,840
1.1 Chemical/biological: markers, dyes, beads	1,200	
1.2 Optics: filters, interference, notch, mirrors,	1,000	
1.3 Bacteria: biological simulant.	640	
1.4 Laser: Gas Laser	2,000	

Publication Costs/Documentation/Dissemination: Provide an explanation of items included in this amount and the per item cost (e.g., the estimated number of pages and the per page cost.)

No cost

Consultant Services: For each consultant, provide an agreement which includes the following information: (1) the services to be provided; (2) the period of performance and the consultant's availability; (3) the qualifications of the consultant to perform the work; and (4) the rate of pay (not to exceed \$453 per day). (Consultants should be chosen using a competitive selection process; otherwise, sole source justifications should be maintained.)

3. CONSULTANT SERVICES 1st YEAR				\$7,950
3.1 Douglas A. Christensen, Ph. D. (EE/Bio. Elec. Engr)	16hrs	\$75/hr	1200	

	specific work in Bioengineering, Laser technology, review			
3.2	James J. Smith, Ph. D. (Microbiologist)	66hrs	\$75/hr	4950
	Experimental Design and review			
3.3	Warrie Means, Ph. D. (Meat Scientist)	24hrs	\$75/hr	1800
	testing meat samples and review			

3.CONSULTANT SERVICES 2 nd YEAR					\$7,950
3.1	Douglas A. Christensen, Ph. D. (EE/Bio. Elec. Engr)	12hrs	\$75/hr	900	
	specific work in Bioengineering, Laser technology, review				
3.2	James J. Smith, Ph. D. (Microbiologist)	66hrs	\$75/hr	4950	
	Experimental Design and review				
3.3	Warrie Means, Ph. D. (Meat Scientist)	28hrs	\$75/hr	2100	
	testing meat samples and review				

Each consultant will work not more than 6 hours in any day (\$450) which does not exceed \$453. The original proposal includes their letter of commitment and a resume which demonstrates their qualifications as a consult on this proposal.

Subawards: For each subaward agreement provide (1) the services to be provided, the names of individuals expected to perform the work, and the expected level of effort for each individual; (2) cost information broken out by cost category (salaries, fringe, travel.

Subaward to the University of Wyoming

Personnel:

A. SENIOR PERSONAL: PI/PD 1 st YEAR			Period of performance	Funds
1	Dr. Paul E. Johnson PD	Direct the subcontract activities	3 months of activity over 1 year	0
2	(To be hired) Engineer	Provide mechanical and electronic design for the Cytometer, controls, and tests of the cytometer system	9 months of activity over 1 year	\$21,016

B. OTHER PERSONNEL 1 st YEAR			
3.	GRADUATE STUDENT	560 hours	\$9,000
Assist the Engineer particularly with the testing of system			
4.	UNDERGRADUATE STUDENT	714hours	\$5,000
Carry out work assigned and work with the engineer and graduate student			

G. OTHER DIRECT COSTS 1 st YEAR			
1.	MATERIALS AND SUPPLIES		\$7,000
1.1	Mech .& Optical Shop (design & construction of cytometer, optics and controls)	280 hrs \$5,600	
1.2	Graduate student tuition (requirement of the University of Wyoming)	\$1,400	

A. SENIOR PERSONAL: PI/PD 2 nd YEAR		Period of performance	Funds
1	Paul E. Johnson Direct the subcontract activities	3 months of activity over 1 year	0
2	(To be hired) Provide mechanical and electronic design for the Cytometer, controls, and tests of the cytometer system	12 months	\$28,020

B 4.	UNDERGRADUATE STUDENT 2 nd YEAR	714 hours	\$5,000
	Carry out work assigned and work with the engineer and graduate student		

G .	OTHER DIRECT COSTS 2 nd YEAR		
1.1	Mech .& Optical Shop (design & construction of cytometer, optics and controls)	280 hrs	\$5,600

For Year 1 and Year 2, Fringe Benefits: FICA 7.65%; Unemployment 01.5%; workers comp., Health insurance, dental care, life insurance, eye glasses, other reasonable and necessary medical procedures; receipts required; The total for all these benefits may not exceed 28% of wages.

The total budgets for the subaward are shown in the first and second year budgets in the original proposal.

Indirect Costs: Provide a current indirect cost rate agreement negotiated by a Federal Agency. In the absence of a current negotiated rate agreement, provide an indirect cost proposal and the related financial statements (income statement and balance sheet) for the two most recently completed annual accounting fiscal periods. If an organization has not had financial activity for these periods, indirect cost proposals should be based on what activities the organization expects during the proposed award period. (See sample indirect cost proposal found at Appendix 6 of the Guide.)

Indirect Costs: 25% on total direct cost less subaward.
10% on subaward
7% Fee on Total direct cost plus Over head

In order to avoid delays in the review and processing of your proposal, please send the requested information within 15 days of the above date to:

To: National Science Foundation
Division of Contracts, Policy and Oversight
Cost Analysis and Audit Resolution Branch
4201 Wilson Boulevard, Room 475
Arlington, VA 22230
Cc: to Bruce Hamilton

Questions related to Items 1 and 2 should be directed to the Division of Grants and Agreements (Attn: Andrea Kline) at 703-306-1212. Questions related to Items 3 through 5 should be directed to the Cost Analysis and Audit Resolution Branch (Attn: Patricia Farrell) at 703-306-1244.

Status: U
Date: Fri, 01 Nov 2002 15:12:05 -0700
From: "Paul E. Johnson" <PJohnson@uwyo.edu>
Subject: FW: letter rpt #2
To: bales@mbj-law.com
Thread-Topic: letter rpt #2
Thread-Index: Ab/8vcHK0sa5GWipEdSErWCqAN2PpYUKawmQAAAbesA=
X-Message-Flag: Follow up
X-OriginalArrivalTime: 01 Nov 2002 22:12:05.0465 (UTC)
FILETIME=[B6429C90:01C281F3]

-----Original Message-----

From: Paul E. Johnson
Sent: Friday, November 01, 2002 3:09 PM
To: Paul E. Johnson
Subject: FW: letter rpt #2

-----Original Message-----

From: Richard W. Shorthill [<mailto:shorth@eng.utah.edu>]
Sent: Wednesday, August 02, 2000 2:13 PM
To: Bruce Hamilton; Paul E. Johnson
Subject: letter rpt #2

Bruce:

Attached is our planning document. Paul still plans to visit Becton Dickenson in about a month and go over our research plans as per the attachment. Our consultants are reviewing our planning document. We have obtained a sample of the "Jack-In -The-Box" Hamburger sample with E.coli 0157:H7 and our microbiologist will be working with it.

Dick

PS; Hope you can read the attachment.

Richard W. Shorthill, Ph. D.
University of Utah
Mechanical Engineering
50 S. Campus Drive
Salt Lake City, UT 84124-9208
UU Office E-mail: shorth@eng.utah.edu
UU Phone: 801-581-8623
UU FAX: 801-585-982

Home E-mail: srw4@uswest.net
Home Phone: 801-278-7042

Content-type: application/applefile; name=plan1.doc
Content-disposition: attachment; filename=plan1.doc
Content-description: plan1.doc

plan1.doc

Brief letter report #2:

STTR Phase II: "Early Detection and Identification of Individual Pathogenic Microorganisms in Food with a Flow Cytometer"

Planing outline

Work Breakdown for June 15th 2000 to June 15th 2001.

Task 0 Administrative Tasks (complete by August 15th)

- | | |
|---|------|
| 0.1 Hire accountant. | Done |
| 0.2 Develop bookkeeping system. | Done |
| 0.3 Develop and implement time recording system. | Done |
| 0.4 Develop reporting and lab notebook system. | |
| 0.5 Develop budget reporting system. | |
| 0.6 Decide on project management software and purchase. | |
| 0.7 Rent office space. | Done |
| 0.8 Purchase computers. | Done |
| 0.9 Network computers. | |
| 0.10 Purchase and install computer software. | |
| 0.11 Develop company web site. | |
| 0.12 Issue press release. | |
| 0.13 Develop hiring plan. | |
| 0.14 Develop fringe benefit plan. | |
| 0.15 Purchase insurance. | |
| 0.16 Develop travel policy and forms. | |

Task 1 Review the Optimal Experimental Configuration for the Demonstration Prototype (complete by August 30)

- 1.1 Review w Warrie Means & Jim Smith USDA requirements for ground beef analysis.
- 1.2 Review w BD USDA requirements for ground beef analysis (August 18 meeting).
- 1.3 Review overall performance criteria (e.g. size, speed, Ease of use, sensitivity, volume throughput, Rate of false positives, detection efficiency).
- 1.4 Review proposed preliminary design and experiment design w consultants & Students
- 1.5 Produce revisions to preliminary designs
- 1.6 Refine WBS based on revision.

Task 2 Build Multi-color Demonstration Prototype (complete by December 15, 2000)

- | | |
|--|--------------------------|
| 2.1 Build rectangular flow cell | Done |
| 2.2 Improve fabrication technique for flow cell | Background task |
| 2.3 Increase N.A. of optics | RWS review N.A. argument |
| 2.4 Incorporate faster CCD camera to permit faster flow | |
| 2.4.1 Obtain camera specs from Electrim | |
| 2.4.2 Decide on camera model & purchase | |
| 2.4.3 Contract for TDI software drivers | |
| 2.4.4 Contract to RSI for IDL software mods | |
| 2.4.5 Modify SoftRay software for fast camera | |
| 2.4.6 Modify SoftRay software for 2-colors | |
| 2.4.7 Test SR software | |
| 2.5 Conversion of data acquisition software to faster C++ code. | (Break this down.) |
| 2.6 Addition of mirror/dichroic combination to divide the image of flow cell into two. | |
| 2.6.1 Design preliminary test version of optics w CCD camera, but wo flow. | |
| 2.6.2 Order parts. | |
| 2.6.3 Construct apparatus on optical breadboard. | |
| 2.6.4 Test apparatus. | |
| 2.6.5 Integrate system w flow cell and two illumination sources | |

Plan 1

(LED or diode laser).

- 2.7 Develop and test LEDs as illumination sources (red and green). (Test with microscope and florescent beads.) **(Optional)**

- 2.7.1 Buy LEDs and optics.
- 2.7.2 Design and fabricate illumination system to be integrated with microscope.
- 2.7.3 Measure power of illumination sources using laser power meter.
- 2.7.4 Compare LED illumination sources with conventional low power laser using Olympus microscope.

Task 3 Perform Measurements using Florescent Spheres (December 15th on)

- 3.1 Determine which spheres to purchase & set up protocol for S/N tests and efficiency of detection tests
- 3.2 Purchase spheres
- 3.3 Run spheres through 1-color system for calibration, S/N analysis, and efficiency analysis
- 3.4 Repeat 1-color S/N analysis and efficiency analysis from Phase I using existing software and new C software **after January 1st.**
- 3.5 Determine protocol for S/N test to improve & optimize for best detection efficiency.
- 3.6 Execute protocol from previous step.
- 3.7 Modify design based on 3.6.

Task 4: Measurements w E. coli.

- 4.1 Modify lab for bio experiments, purchase necessary supplies, and determine lab safety and notetaking protocols. **Done**
- 4.2 Order and begin culturing E. coli. **Done**
- 4.3 Perform 1-color testing.
 - 4.3.1 Determine labeling protocol **for 1-color measurements.**
 - 4.3.2 Test protocol on O157:H7 and benign E. coli to determine scope of problems with cross-reactivity. (Use epifluorescent microscope.)
 - 4.3.3 Make necessary corrections.
 - 4.3.4 Label E. coli (benign, live) and count w microscope & flow cell for comparison.
 - 4.3.5 Perform detection efficiency calculation.
 - 4.3.6 Review.
 - 4.3.5 Label E. coli (benign, dead) and count w microscope & flow cell for comparison.
 - 4.3.6 Perform detection efficiency calculation.
 - 4.3.7 Review.
 - 4.3.8 Label O157:H7, dead and count (before or after killed?).
 - 4.3.9 Perform detection efficiency calculation.
 - 4.3.10 Determine protocol for mixed O157:H7/benign E. coli (all dead? Or just O157) mixed sample to determine cross-reactivity.
 - 4.3.11 Count mixed samples to determine cross-reactivity (rate of false positives).
 - 4.3.12 Review.
 - 4.3.12.1 Determine protocol for counting E. coli in ground beef spiked w O157 **after Jan.1.**
 - 4.3.12.2 Benign
 - 4.3.12.3 O157
 - 4.3.12.4 mixed E. coli
 - 4.3.12.5 Review
 - 4.3.13 Count E. coli in ground beef spiked w O157
 - 4.3.13.1 Run sample in sterile ground beef with no E. coli
 - 4.3.13.2 with benign E. coli, unlabelled
 - 4.3.13.3 benign labeled
 - 4.3.13.4 O157, labeled
 - 4.3.13.5 Mixed, labeled
 - 4.3.13.6 Calculate efficiencies, false negatives, false positives

4.3.13.7 Review

- 4.4 Perform 2-color testing
 - 4.5.1 Determine experimental design
 - 4.5.2 Label E. coli (benign) and count w microscope & flow cell for comparison.
 - 4.5.3 Perform detection efficiency calculation.
 - 4.5.4 Review.
 - 4.5.5 Label O157:H7 and count.
 - 4.5.6 Perform detection efficiency calculation.
 - 4.5.7 Determine protocol for E. coli mixed sample to determine cross-reactivity.
 - 4.5.8 Count mixed samples to determine cross-reactivity (rate of false positives).
 - 4.5.9 Review.
 - 4.5.10 Determine protocol for counting E. coli in ground beef spiked w
 - 4.5.10.1 O157:H7
 - 4.5.10.2 Benign
 - 4.5.10.3 mixed E. coli
 - 4.5.11 Count E. coli in ground beef spiked w
 - 4.5.11.1 O157:H7
 - 4.5.11.2 Benign
 - 4.5.11.3 Mixed
 - 4.5.12 Review

High risks tasks:

- 1. Build flow tube.**
- 2. Label O157 and test vs. benign.**
- 3. Separate pathogen from ground beef.**

EXHIBIT G

Kristin J. Bobbitt

From: Paul E. Johnson
Sent: Friday, July 21, 2000 11:37 AM
To: 'jim00@in-tch.com'; 'shorth@eng.utah.edu'; Warrie J. Means; Kristin J. Bobbitt;
'sundance@uwyo.edu'; 'lunds2@uwyo.edu'
Subject: Work Breakdown



WBS.htm

SoftRay team members,

I have attached (in html) a first-draft work breakdown for the first year of our NSF Phase II. Please review it and give me modifications/comments, as well as indicate to me where you would like to participate. I will attempt to put in dates in the next version. This WBS will serve as the basis for weekly task review meetings and biweekly email progress reports to the NSF.

Please note that each line should be no longer than two weeks in time. Otherwise it needs to be broken into subtasks. Some of the tasks that I have listed here might not be realistically completed in two weeks.

You will need to refer to the original proposal in order to adequately interpret this WBS.

I would like to have this plan finished within two weeks in order to begin interaction with Becton Dickinson. I meet with BD in San Jose on August 18th.

I can't tell you how excited I am that this project is now underway.

Thank you,

Paul

Work Breakdown for June 15th 2000 to June 15th 2001.

Highlighted tasks are to be started after January 1, 2001. Any single line that will take over two weeks should be broken into subtasks, each of which should take less than two weeks.

Task 0 Administrative Tasks (complete by August 15th)

- 0.1 Hire accountant. Done
- 0.2 Develop bookkeeping system.
- 0.3 Develop and implement time recording system.
- 0.4 Develop reporting and lab notebook system.
- 0.5 Develop budget reporting system.
- 0.6 Decide on project management software and purchase.
- 0.7 Rent office space.
- 0.8 Purchase computers. Done
- 0.9 Network computers.
- 0.10 Purchase and install computer software.
- 0.11 Develop company web site.
- 0.12 Issue press release.
- 0.13 Develop hiring plan.
- 0.14 Develop fringe benefit plan.
- 0.15 Purchase insurance.
- 0.16 Develop travel policy and forms.

Task 1 Review the Optimal Experimental Configuration for the Demonstration Prototype (complete by August 30)

- 1.1 Review w BD USDA requirements for hamburger analysis.
- 1.2 Review w Warrie Means & Jim Smith USDA requirements for hamburger analysis.
- 1.3 Review overall performance criteria (e.g. size, speed, Ease of use, sensitivity, volume throughput, Rate of false positives, detection efficiency).
- 1.4 Review proposed preliminary design w consultants & Students
- 1.5 Produce revisions to preliminary design
- 1.6 Refine WBS based on revision.

Task 2 Build Multi-color Demonstration Prototype (complete by December 15, 2000)

- 2.1 Build rectangular flow cell Done
- 2.2 Improve fabrication technique for flow cell Background task
- 2.3 Increase N.A. of optics RWS review N.A. argument
- 2.4 Incorporate faster CCD camera to permit faster flow
 - 2.4.1 Obtain camera specs from Electrim
 - 2.4.2 Decide on camera model & purchase
 - 2.4.3 Contract for TDI software drivers
 - 2.4.4 Contract to RSI for IDL software mods
 - 2.4.5 Modify SoftRay software for fast camera
 - 2.4.6 Modify SoftRay software for 2-colors
 - 2.4.7 Test SR software
- 2.5 Conversion of data acquisition software to faster C++ code. **(Break this down.)**
- 2.6 Addition of mirror/dichroic combination to divide the image of flow cell into two.
 - 2.6.1 Design preliminary test version of optics w CCD camera, but no flow.
 - 2.6.2 Order parts.
 - 2.6.3 Construct apparatus on optical breadboard.
 - 2.6.4 Test apparatus.
 - 2.6.5 Integrate system w flow cell and two illumination sources (LED or diode laser).

- 2.7 Develop and test LEDs as illumination sources (red and green). (Test with microscope and florescent beads.) **(Optional)**
 - 2.7.1 Buy LEDs and optics.
 - 2.7.2 Design and fabricate illumination system to be integrated with microscope.
 - 2.7.3 Measure power of illumination sources using laser power meter.
 - 2.7.4 Compare LED illumination sources with conventional low power laser using Olympus microscope.

Task 3 Perform Measurements using Florescent Spheres (December 15th on)

- 3.1 Determine which spheres to purchase & set up protocol for S/N tests and efficiency of detection tests
- 3.2 Purchase spheres
- 3.3 Run spheres through 1-color system for calibration, S/N analysis, and efficiency analysis
- 3.4 Repeat 1-color S/N analysis and efficiency analysis from Phase I using existing software and new C software after January 1st.
- 3.5 Determine protocol for S/N test to improve & optimize for best detection efficiency.
- 3.6 Execute protocol from previous step.
- 3.7 Modify design based on 3.6.

Task 4: Measurements w E. coli.

- 4.1 Modify lab for bio experiments, purchase necessary supplies, and determine lab safety and notetaking protocols. Done
- 4.2 Order and begin culturing E. coli. Done
- 4.3 Perform 1-color testing.
 - 4.3.1 Determine labeling protocol for 1-color measurements.
 - 4.3.2 Label E. coli (benign) and count w microscope & flow cell for comparison.
 - 4.3.3 Perform detection efficiency calculation.
 - 4.3.4 Review.
 - 4.3.5 Label O157:H7 and count.
 - 4.3.6 Perform detection efficiency calculation.
 - 4.3.7 Determine protocol for mixed O157:H7/benign E. coli mixed sample to determine cross-reactivity.
 - 4.3.8 Count mixed samples to determine cross-reactivity (rate of false positives).
 - 4.3.9 Review.
 - 4.3.10 Determine protocol for counting E. coli in hamburger spiked w after Jan. 1
 - 4.3.10.1 O157:H7
 - 4.3.11 benign
 - 4.3.12 mixed E. coli
 - 4.3.11 Count E. coli in hamburger spiked w
 - 4.3.11.1 O157:H7
 - 4.3.11.2 Benign
 - 4.3.11.3 Mixed
 - 4.3.12 Review
- 4.4. Perform 2-color testing
 - 4.4.2 Label E. coli (benign) and count w microscope & flow cell for comparison.
 - 4.4.3 Perform detection efficiency calculation.
 - 4.4.4 Review.
 - 4.4.5 Label O157:H7 and count.
 - 4.4.6 Perform detection efficiency calculation.
 - 4.4.7 Determine protocol for mixed O157:H7/benign E. coli mixed sample to determine cross-reactivity.
 - 4.4.8 Count mixed samples to determine cross-reactivity (rate of false positives).
 - 4.4.9 Review.
 - 4.4.10 Determine protocol for counting E. coli in hamburger spiked w
 - 4.4.10.1 O157:H7

- 4.4.11 benign
- 4.4.12 mixed E. coli
- 4.4.11 Count E. coli in hamburger spiked w
 - 4.4.11.1 O157:H7
 - 4.4.11.2 Benign
 - 4.4.11.3 Mixed
- 4.4.12 Review

EXHIBIT H

Sept 4 Holiday

Sept 5 met w Julie Kellogg regarding
use of LEDs in flow

met w Mike Lund re
fixing leak in flow cell

Sept 6 met w Mike Lund

Sept 7 Weekly Retcon: w only } 2 hours
Duke & Paul

Aug Revised WBS
Wrote up minutes
Worked on expense account

Telecon w Winslow

Winslow suggested not signing
any agreement w BD that gives them
first right of refusal until they have
made a monetary commitment.

Oct 4, 2000 Wed

15

Sept. 21

Telecon: Dick, Paul, Jerome, Michael, Kristen

1. Jerome: server will be up soon with when IP is up. Used for package.
2. Michael: right page antibody staining test on Saturday CCD dark current
- A.I. - camera manual
3. microbiologist being hired
4. flow cell is being tested on Sat. with "breaking barriers"
5. Web site nearly complete

Completed Biohazard safety meeting w Michael. Need Hep B shot

Finished changing domain for www.softway-inc to www.concentra.com

met w Julie re: LED testing.

Called Jim Smith:

A.I. before we choose labels do detox spectrofluometry of beef washings

Sept. 22

Arranged with www.vconference.com for conference calls at \$25 per call (\$99/month for one call a week plus \$25 for each additional. Prepaid by credit card.

met w Michael

Read Cytometry article from July 1, 2000 on triple label for viability + antibody,

Sept 25

Oct 4, 2004
JLB

17

Prepared for telecon.

Telecon w BD Bateman (Tom Wamund, Jim Smith, Lunch, & Mondar Nagar)

See minutes in 3-ring binder.

Sent out minutes - of meeting.
Met with Harvey re 1080.

Telecon w Krissy Manion & Susan Epelko
(BD)

~~Met~~ Recommendation to make agreement
with Univ. of Utah.
Sign off on tech transfer
office.

AI < Copy of letter to Univ. of Shorthill

AI < Background intellectual property in
final agreement w UW.

AI < Send draft of Shorthill
agreement to BD.

Posted job ads in Mole Ag & Animal Sci.

Sept 26

Met w Julie re LEDs.

Worked on revised update & sent to
Bob Hoffman. (Updated w
his comments). See notebook
copy.

Sept. 27th

Interviewed Amanda for job. (in Bio.)
Faxed CV to Deb (Amanda.)

Oct 25

Met w Amanda. She is looking at installing eye wash & swapping lab fawcetts.

Met w Michael

Met w Jerome and discussed how to use bugs remaining in C++ Browser.

Worked on travel and expenses.

Met w Rustin. Will start money market today. W. Hask. Made to work on disrepair. Worked on time sheet. DIPS still.

Talked to Curtis. Work order submitted for card lock in pathogen room.

Oct 26

Weekly Conference

Amanda, Jerome, Julie, & Michael (w me)

New action items

1. Talk to Warren re: ConAgra visit. Paul
2. Build rectangular LED beam optics. Julie
3. Test LED on scope w beads. Julie
4. Ask Warren about ConAgra team. Paul
5. Spend 2-3 hours on C++ software. Jerome & Paul
6. Move equipment. Everyone.
7. Ask Smith about BAM, AOAC manual. Paul

Met w Julie. Gave her copy

of Don Klipstein's LED main page:

<http://www.misty.com/~don/ledx.html>

See also ledmuseum.home.att.net

Suggested that we try semi-cylindrical lens for rectangular (~2mm x 200µm) beam.

Nov 14

28

A

Interviewed 2hr twice.

Paul E. Johnson

From: Paul E. Johnson
Sent: Tuesday, November 14, 2000 3:34 PM
To: 'julee@uwyo.edu'
Cc: 'shorth@eng.utah.edu'

Julie,

I talked to a physicist at Becton Dickinson. He said that they've actually been able to get more intensity out of the blue Nichia diodes running between 30 and 40 ma (although they might not run that hard forever).

Isn't your experience that the blue diodes fatigued at 30 ma like the green ones?

Bob suggested that the flat packs will probably dissipate heat better than the lensed diodes, so that we should be able to drive them harder.

Paul

Nov 15 Spent ~~to time~~ in
lab helping reorganize.

2 hours on USDA proposal.

Nov 16 SR Meeting. (see
A-I list). Worked on USDA proposal
lab. Worked on swipe lock for

Nov 17 Worked with Julie on
setting up CCD w microscope.
Electron scanning on microscope
doesn't work.

Talked to Amanda re antibodies.

Feb. 20th

Dennis + I talked to Kussig + Noel re: contract. It looks like no up front money, but we will try to get foreign patent costs.

Amanda has scoured out larger centrifuge.

Jon Wardlaw has recommended a possible source of ~~mouse~~ polyclonals.

Email exchange: Dehua is testing C++ program code.

Fixed 2-page LED brochure to David met w Amanda.

2/21st

Emailed Jon W. Suggested that time is too short to do polyclonals. Talked to Amanda re antibody selection.

Letter of Engagement sent from UW to Jennifer. Worked w Julie on LED/microscope.

2/22nd

SR Meeting
Aubrey

Email from Sympson re Edgewood. Travel to reach John Pore re: contract. UW / Blue Sky.

Feb 23

Met and worked w Julie on LEDs. Met Amanda.

3/26

Met w Amanda re 04157:FF?
medium
email xchg w Jerome & Julie
re LEDs

3/27

email xchg w Kissing Manion
re contract
met w Amanda

3/28

negotiation mtg. w Longiulli
Met w Amanda
sent email to D. Cook re: negotiations

3/29

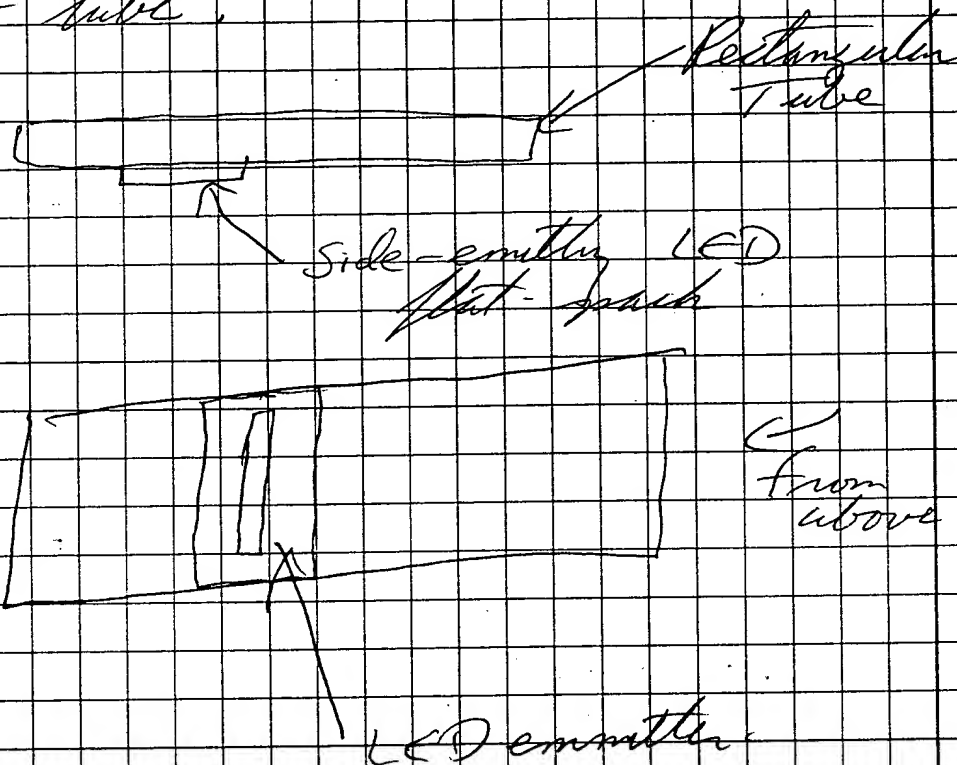
source of blue LEDs: www.hosfelt.com/leds/ (led)
Worked on Travel & Time
Longiulli emailed Bern & Roger re:
negotiations

3/30

met w Jerome
Time Sheets

3/30

My idea for LED illumination of flow tube. D



can glue (optical cement) more than 1 LED to tube to achieve highest throughput.

I asked Jerome to order R-PE beads to illuminate with blue and/or green LEDs.

4/2/01

met w Kristen to sign checks.

4/3/01

met w Jerome re LED Hummich
met w Amanda

4/4/01

met w Jerome re LED Hummich

4/5/01

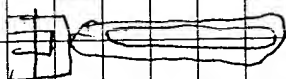
SoftRay mtg - 2 hours

4/6/01

met w Amanda

called Hoffman / no answer

called Jim Smith

worry about muscles absorbing
label \Rightarrow may be injected into
ground beef, rocket fuelget bacteria for cross-reactivity
from vaccine wash

LED contacting tubing

Paul E. Johnson

From: Paul E. Johnson
Sent: Friday, April 13, 2001 12:37 PM
To: 'shorth@eng.utah.edu'; Asprey, Bill; Asprey, William; Kellogg, Julie; Kristin Bobbitt; lunds2@uwoy.edu; sundance@uwoy.edu; Votaw, Amanda; Zhao, Dehua
Subject: New Ideas!

Everyone,

I just talked to Bob Hoffmann at BD and gave him a verbal update on our progress. He made some terrific suggestions:

1. Illumination Sources: He suggested that we think about illuminating with laser illumination run through a multi-mode fiber to make it non-coherent. Even better-- LumiLeds makes LEDs that put out 100mW!! They have emitting regions that are 1mm x 1mm. He suggests collimating this light with Kohler (with an unlaut) optics, that are used in microscopy to collimate light as well as to make the beam uniform. Then we could illuminate from above, at an angle, onto a 2mm x 1mm patch, or whatever, uniformly. Illumination from above through 100 microns of material would also alleviate absorption by the medium. Hoffman is sending a 505 nm LED for us to evaluate. He suggests replacing our microscope illuminator with this to test it.
2. Labels: If we have to illuminate in the red, because of solution opacity, Bob suggests APC, CY5, or one of the Molecular Probes Alexa dyes. He wanted to know whether our meat solution appears red, and how cloudy it appears?
3. Beads: If we want to experiment with bicolored beads for our 2-color measurements, he suggested talking to Ken Davis, who just happens to be developing two colored beads (based on attaching antibody to the beads, labeled with two different labels).
4. Detergent: If we need to add detergent to our disgusting beef cocktail, to keep miscles that absorb dye from being detected as bacteria, he suggests talking to Ken about the possible adverse effects that detergent will have on labels. Some labels can bind to detergent, others can react in other adverse ways.

An enormously profitable discussion!

Paul

EXHIBIT I

4/7/01

1. telecon w. Legoyte. They appear to have IRAS technology in patent.

2. Andy Seneca (Watuk) telecon.

mentioned Ferkigard + Kerry antibody
(Too expensive)
Biosensors

They are working on separation + recovery from food. CRADA!!
Fullerene!!
We could be funded from Vetcon.
Demo in Sept.?

Talk to Janet. Tensen@sbccom.apsec.
army.mil re water

Interested in rapid digester for
food. Easy to use

Field studies. Portable

Have

Book 3 P.T.

SIGNATURE	DATE	WITNESS/TA	DATE
<i>[Signature]</i>	11/14/01		

EXP. NUMBER	EXPERIMENT/SUBJECT	LOCKER/DESK NO.	COURSE & SECTION NO.
NAME			

4/7/01

Time sheet.

SIGNATURE	DATE	WITNESS/TA	DATE
<i>[Signature]</i>	4/6/01		

NOTE: INSERT PERIODIC TABLE UNDER COPY SHEET BEFORE WRITING • THE HAYDEN-McNEIL STUDENT LAB NOTEBOOK

EXP. NUMBER			12
NAME	LOCKER/DESK NO.	COURSE & SECTION NO.	

1. Bioimageinstall.txt (in Dan) describes IDL environment and installation routines.

2. Bioimage.pro data acquisition. compile @test
 set TDI set 900.000 (corresponds to pump run of 15 w/ Dell HP) (3ml?)
 3. PSP3.pro counting
 logfile.txt

Bioimage.pro file preference startup
 set working directory to C:\dan for sub-software (.pro files)

look at bioimageinstall.txt

stat analysis in C:\0105

anum3-8 Corrected.pro - iterated stats
 anum3-8... other one does one stat iteration

A. Bioimage 225000 is probably maximum exposure on Dell (pump rate of 60.)?

B. TDIfirst2.exe (source code in Public) never tried on null sample
 set exposure 30 is 3.0 (use it) \Rightarrow TDI source \Rightarrow dan
 4 spot size (3ml?)

C. Asprey's password is blackdog

D. anum3-8... like short folder names so that they won't wrap.

compile command "pspasp"
 @ test stop
 analysis goes into data. set
 read with excel

SIGNATURE	DATE	WITNESS/TA	DATE
	11/14/81		

NAME	LOCKER/DESK NO.	COURSE & SECTION NO.
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#coauto } outputs from auto iteration version
 #coauto
 #coname }
 multi - it doesn't require stop!

* cut & paste coauto & coname *
 To import to excel

* 1st columns are counts, 2nd overlap. *

* image counts are cumulative!! *

Dehua's software logfile.out goes into Dan
 accumulator. turn w' y for excel

* only need 30 images from runs

LEDs run 350ma couple minutes to
 but output drops over time
 20% drop in output from 325ma

2mm spot size w LED

need to use both sets of lens pairs:
 one for objective & one for LED

Tube red is 10^{10} out of bottle
 that $1/1000$ dilution w BPW

magnetic stir for 30 minutes
 (4 on stir plate / visible vortex)
 sonicate for 2 minutes.

SIGNATURE	DATE	WITNESS/TA	DATE
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August 27

Worked all morning on expenses/
 France.
 met w Amanda
 Worked several hours w Ryan
 Greg Jackson called re renewing
 insurance policy. He asked Kimster
 to take care of this.

Aug 28

met w Amanda.
 Worked w Ryan
 Lab meeting.

Aug 29

Interviewed Jean Jewell
 met w Dennis to get stock shares.
 Worked w Ryan
 Signed up for Venture West Conf.
 + SBIR (S Dakota) reimbursement
 Dropped check by David Janquille
 w Cap. Table + stock shares.
 Emailed Eugene re
 biology month.

Aug 30

Mailed RWS LED and K-1.
 Emailed Debra + Jerome re
 access to lab computer.
 Received email re: new round
 of tainted beef.

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Worked on reports for BD.

August 31

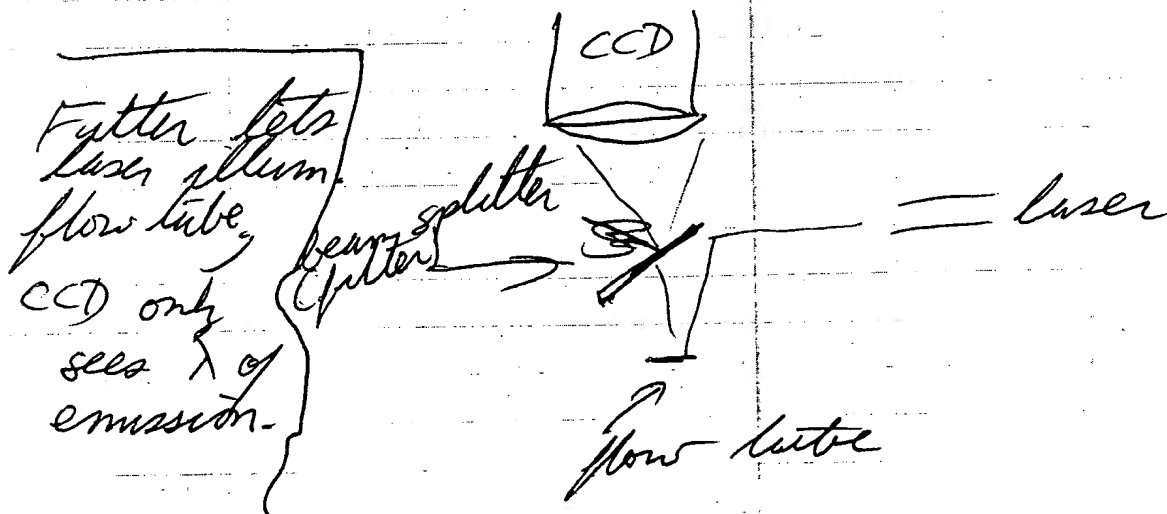
Submitted revised ad for Boomeray.
Submitted (+ worked on) revised new
BD reports.

Sept. 4

Called + sent info to Dan
at Equinox re: LED portion of
project.
Met w Amanda.

Sept 5

Talked to John Miller about
building beam splitter



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DATE

Sept 13.

Lab mtg.

mtg w Amanda.

Sent contract mods to Kussy/BD.
(2 emails).

Sept 14

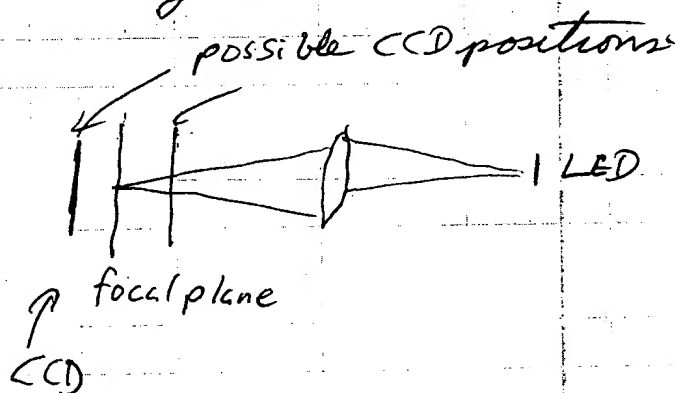
met w Amanda
worked on LED setup

Sept 17

Sent out signed CRADA.

Work on time reports.

Tests (instead) on LED setup,
works great!



$\pm 10\%$ uniform over $2\text{mm} \times 2\text{mm}$ field.

SIGNATURE

DATE

WITNESS/TA

DATE

NAME

LOCKER/DESK NO.

COURSE & SECTION NO.

Paul E. Johnson

From:
Sent:
To:Paul E. Johnson
Monday, September 17, 2001 12:19 PM
Amanda S. Votaw

Amanda,

I tried some more quantitative experiments with my crude LED setup in the lab. The result, after 20 minutes of experimenting, is that it is straightforward, if not trivial, to produce a square beam, 2mm across, that is within 10% of being uniform. I projected the LED beam onto the bare CCD and imaged it, after turning the LED down so far as to be almost off and turning the CCD exposure time down to 1 millisecond. I then produced row plots from the CCD that are pretty uniform. In order to get a large enough uniform beam, I just moved the CCD until the image of the LED was so far out of focus that it produced a uniform beam about 2-3 mm across.

After lunch I'll call LumiLeds and see if they are now shipping blue and cyan leds. If so, I'll order some leds and optics than are less humongous than what I'm using now. After these come in I'll have the machine shop put everything together in a nice aluminum or brass package.

Obviously, if Lumileds is shipping blue/cyan leds, we can use blue-illuminated dyes, such as FITC. In the short term, we can even use FITC.

Not bad for a morning's work, and a lot more fun than bead counting. Maybe I should quit for the day while I'm ahead.

Paul

*Used Olive to try out possible lenses
for LED illumination.*

*try part # 32490 Edmund.
25.00 mm DCX eff 25.00
at a distance ca. 30.0 mm
from LED.*

*Need 532 nm + 488 nm 10nm FWHM
interference filters.*

LEDs \$8.00^{ea}/min of 50 from Future electronics

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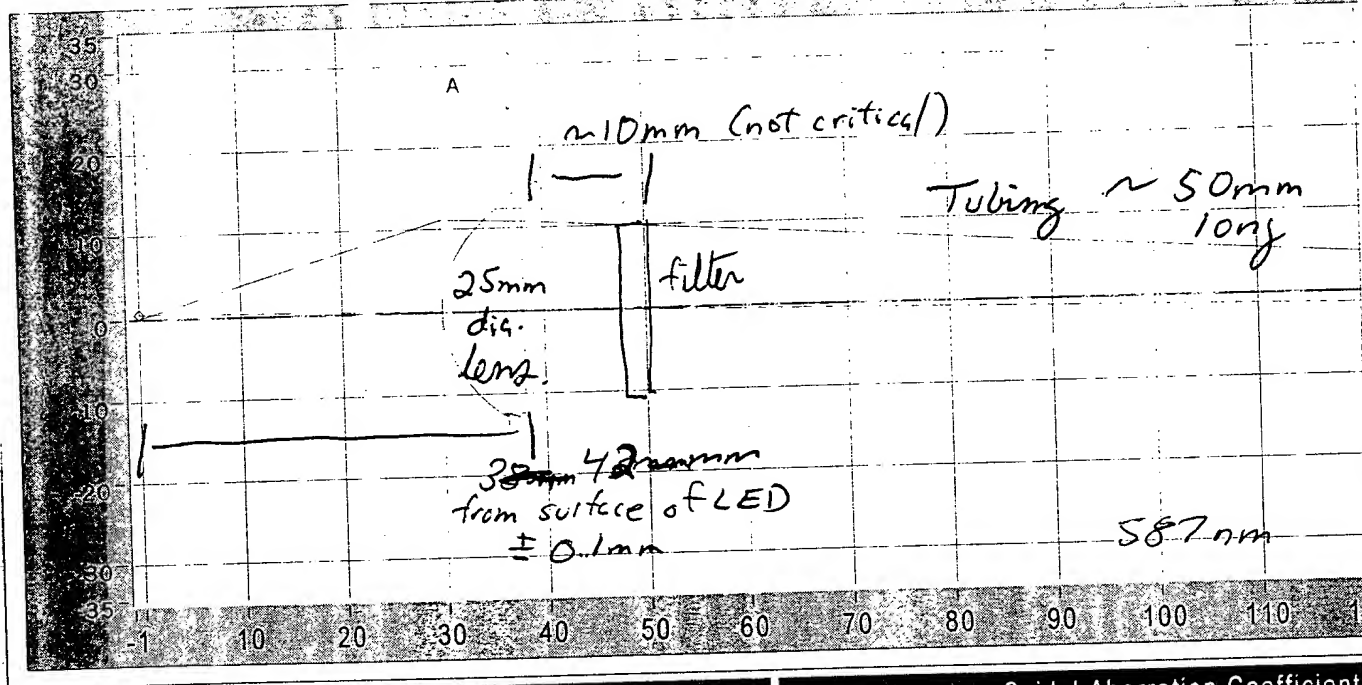
Sept 18

Met w Steve (shops re data system box.
met w Gus/shops re illumination system box.

Tried out older square-board LED.
it is green. Amount #414 0712 49

Lumileds uses same "near optics"
on collimated + non-collimated
optics.

Sept 19



Paraxial Data				
Surface	Z	Y marg.	Y chief	Radius
Object	0.000	0.000	0.500	inf.
1	30.000	11.250	0.000	16.820
2	38.000	10.891	-0.080	inf.
Image	182.951	0.000	-2.496	inf.

Seidel Aberration Coefficients				
Lens	Sph.	Coma	Astig.	P
45098	4.9288	0.0780	0.0014	0
Totals	4.929	0.078	0.001	0

24/36

Sept 19

met w Amanda

Questions on optics

1. How big is LED emission surface? $1\text{mm} \times 1\text{mm}$

2. How far is LED surface from mounting surface?
measure

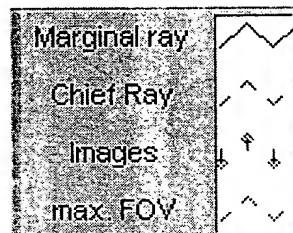
3. A/# of LED emission?
 90°

4. Is using a DCX lens equivalent to a PCX.
In terms of mechanical design. No

5. 2-x mount?

OLIVE /1.0.
see previous page

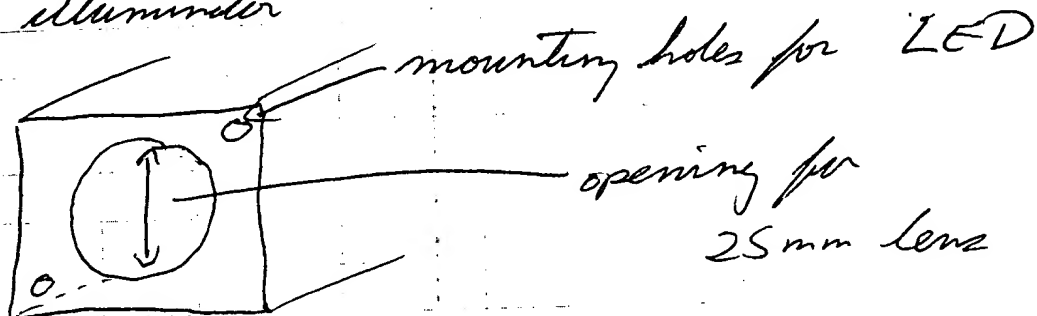
High Chiva
Systems
Tucson AZ
info@highchiva.c
www.highchiva.com



DATA

Object size	0.50
Image size	-2.50
pMag	-4.99
parx. focus	144.95
system len.	182.95
working F#	6.65
Stop Radius	11.25

LED illuminator



On other end mount for 1" filter.

Spent all day on optical design for LED illuminator. Δ negotiate IP w BD (mtg. w Dennis

Cook, telecon w Noel, Kuzin, & Bob Hoffman).

Gave machining job to Gus. Est. time to completion = 1 week.

Could go w aspheric condenser, such as Edmund L43-484.

- ★ Need lens mount!
- ★ Order new broader filters!!!
- ★ X-2 mount.
- ★ Order LEDs.

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DATE

Sept. 20

met w Amanda.

Softway mfg.

Talked to John \Rightarrow tomorrow will be completion of reflective optics. Can do imaging tube next week.

Submitted ad to Boomerang for

Engineer / Tech.

Sept 21

Mark Watron improve consultant.

Looked at www.omegafitters.com
order green LUMI LEDs

test w 488nm interference filter
~~by rose~~ but use omega fitters
(from curvometer) for FITC,
Alexa 488, R-PE.

Consider using aspheric condenser lens to collect more light. Problem w combo of f/# of collecting optics, magnification, & working distance. To keep mag. the same and go to faster optics, working distance must decrease.

Sept. 24

met w Amanda.

worked on USB S proposal 2 hours

worked on camera lens design
(mocked up in lab) 2 hours

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DATE

Oct 1, 2001

Met w Gus

Preliminary try-out of illumination
optus. It works. Next step
is to try even illumination test.

+ DL0157 (SUlass)
apple 3/4 (Bios)

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11/1/01

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DATE

led test 3.gif 9cm from end of illuminator (further than focus)
 to end of camera
 .2 amp thru led
 1 ms inty. time
 $\sim \pm 10\%$ over 2mm

led test 4.gif From 11cm 1ms 1ms
 $\sim \pm 10\%$ over 2mm

led test 5.gif 5cm
 $\sim \pm 10\%$ { maybe much more square!! }

led test 6.gif 7.5cm in focus!

led test 7.gif 6cm in focus!

led test 8.gif 5cm again WONDERFUL!!

led back.gif background image!

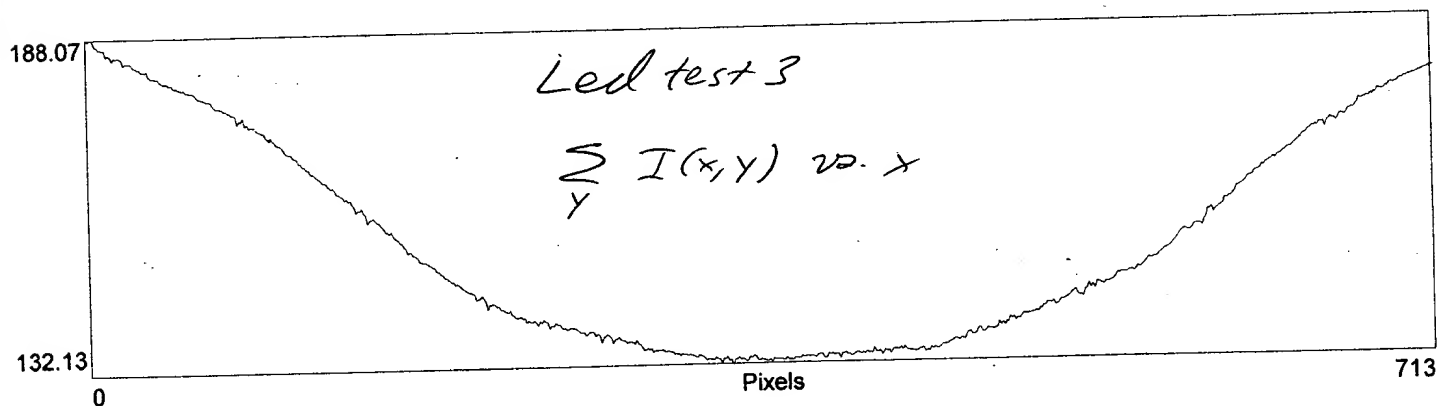
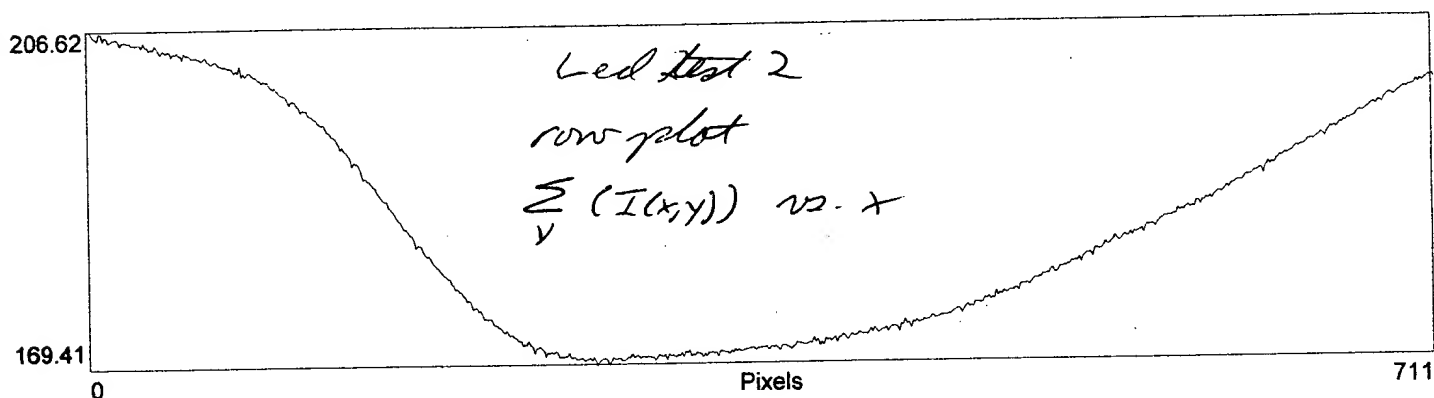
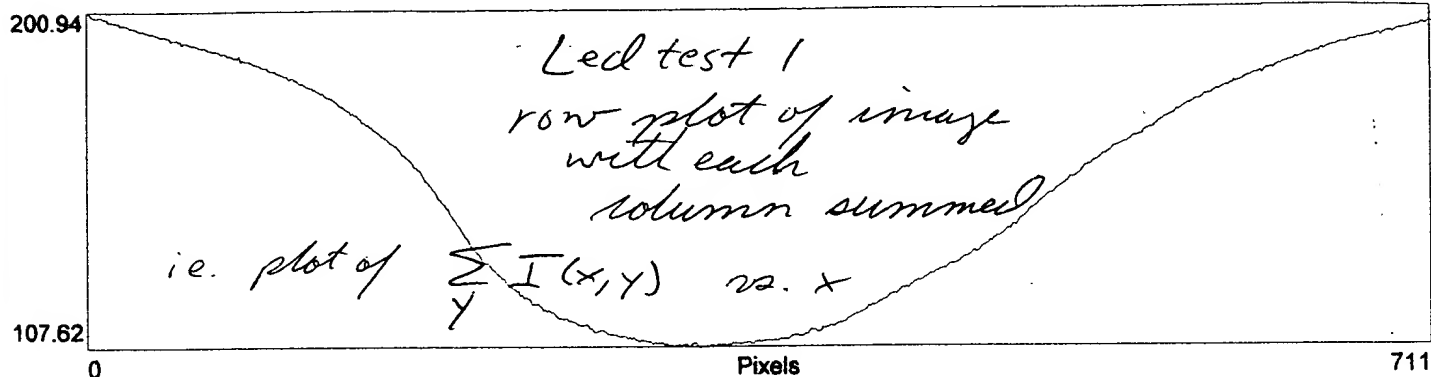
Power w 3.5 amps 2.02 no filter $\sim .4$ amps
 .13 w 532 nm filter
 (interference)

green? LED

3.42 at $\sim .75$ amps no filter

low power due to slow f/# of optics.
 f/2 system, need $\sim f/0.7$ system.
 Losing 90% of light!!!

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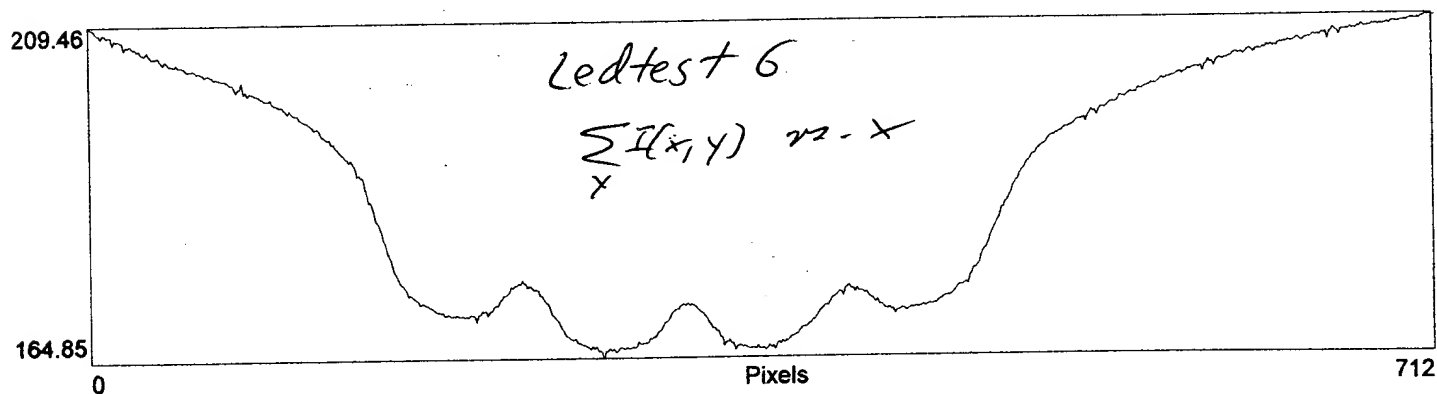
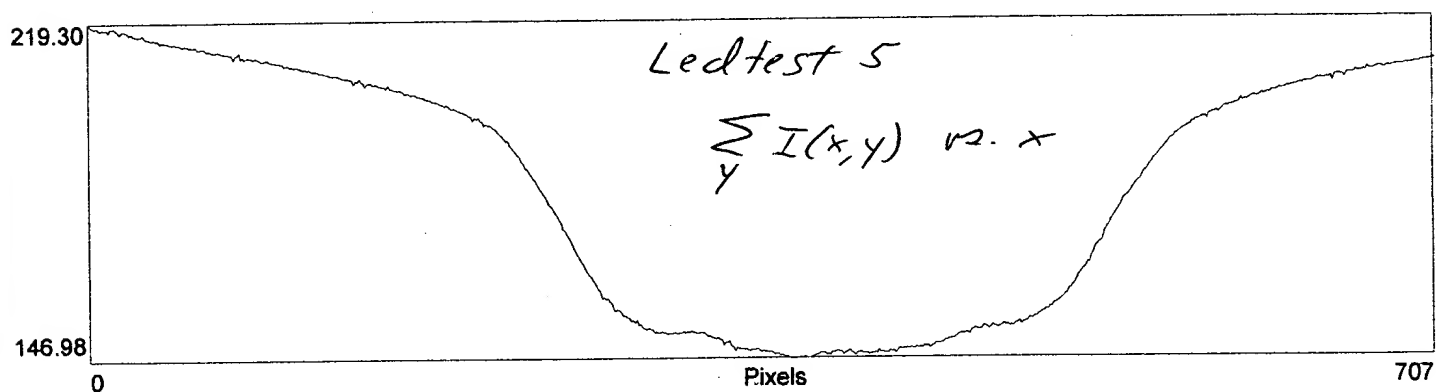
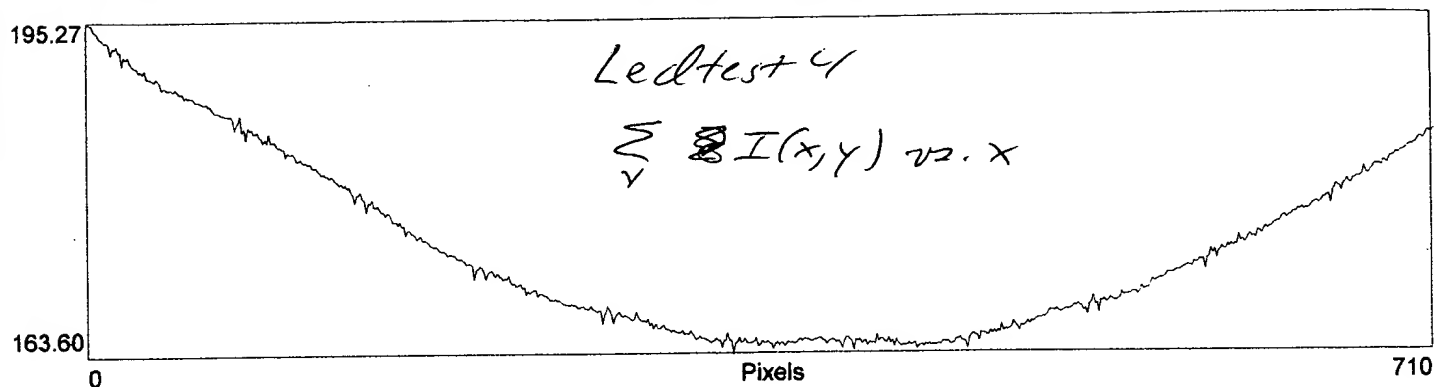
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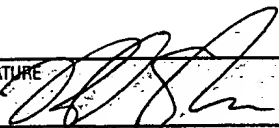
DATE

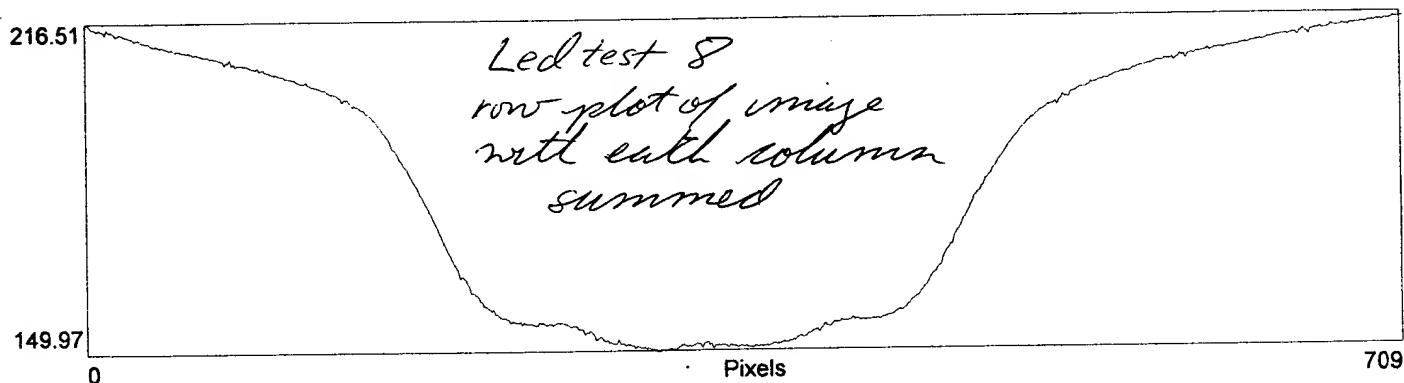
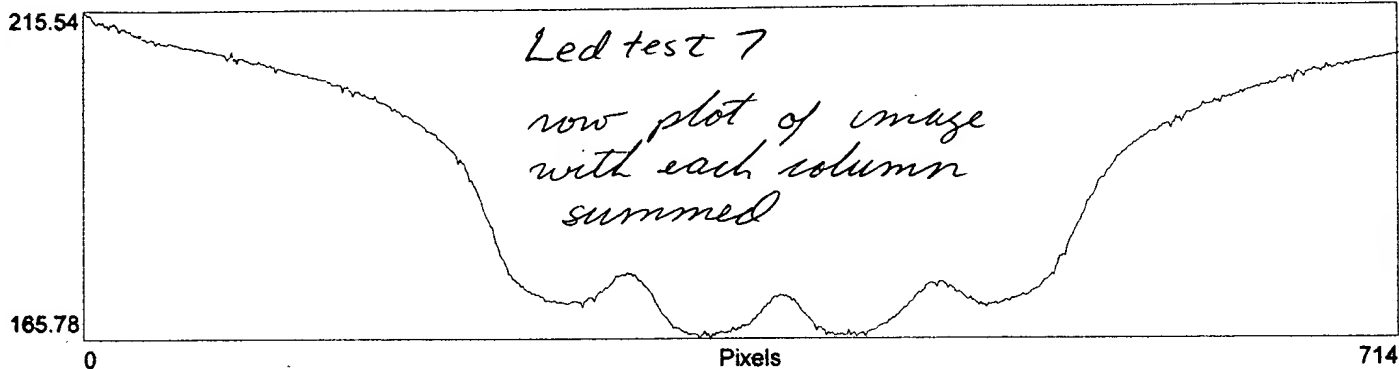
8/6/11/01

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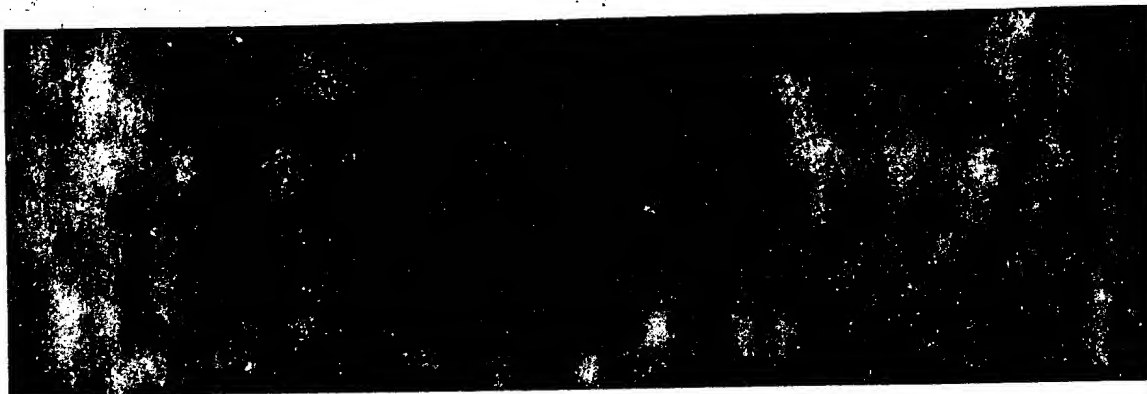


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Led test 5.gif.

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LED test 6.8if

Oct 2

sent email to Dick re LED system

Oct 3

ordered $f/0.5$ lens from Edmund.

Oct 4

Lab meeting.

asked Dan to modify LED illuminator
for ~~LED~~ $f/0.5$ lens.

Played w $f/0.5$ lens.

Dick is sending x-y mover.

Oct 5


corrected illuminator for correct

FL.

Called Allison's rep. Harold Allison
Worked on fixing stornacher.

Oct 7

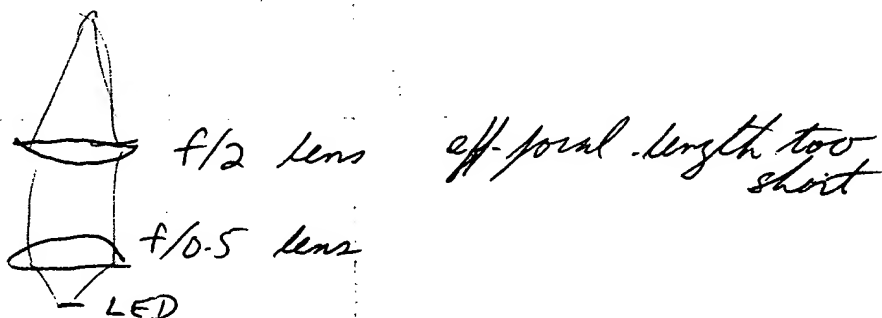
Played with illuminator

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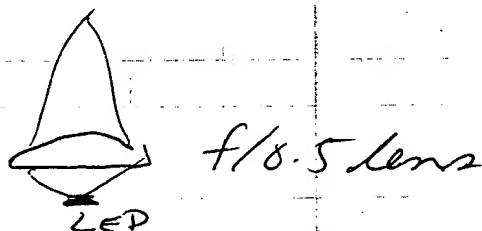
[Oct. 8]

Last week's

Current version of alluminator:



Current version:



350 ma \Rightarrow 5 mW

500 ma \Rightarrow 6 mW

} too small for increase
in speed from f/2
to f/0.5

Try new Cyan LED. Is green LED
worn out?

Clean lens.

New detector?

met w Allison 1 hour.

Played with optics

I talked to Leo Weitzmann re proposals

+ Spincon Sampler (Mid West Research

Institute). www.mri-research.org/led/spincon.html

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Oct 9

email letter from Krissy re BD collab.
not good

Deposit check (Finagra) to SR checking.

Oct 11

Lab mtg. w Allison + Tony.

Investigate filters

Worked on proposal 2 hours.
USGS

Oct 12

Talked to Lee Sylvers. (clairus inc
Rockville MD) sent CDA.

Received filter specs from Ellen

Fenner omega.
Worked on USGS proposal 4 hours.

Order

Oct 14

Ordered filters from Edmund.
650 nm + 700 nm 80mm wide.

Oct 15

Worked 8 hours on USGS proposal.
submitted.

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[Signature]

DATE

10/11/06

WITNESS/TA

DATE

Oct 16.

worked in lab 2 hours w LED.
and new mount.

- 1.) check for 25mm instead of 50mm f.l. lens. ✓ OK.
- 2.) redo beam profile plots ✓ OK
- 3.) perform prelim. bead images
- 4.) test flow tube for flow! ✓ OK
- 5.) have Tony mix FITC + shoot images for photometric test.

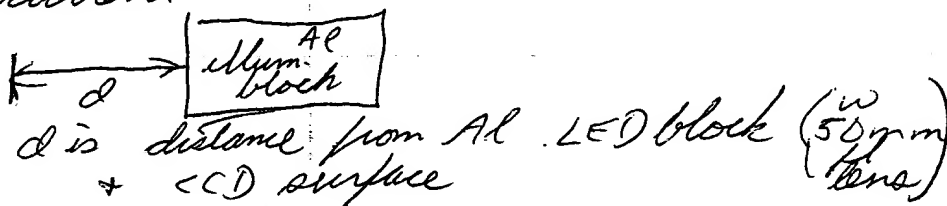
Oct 17

Worked on time sheets.

1 hour.

took images of LED beam (1ms intg. time)
+ low current

1ms intg. time



d is distance from Al LED block (50mm lens)
+ CCD surface

d	image
30mm	leda 30.tif
35mm	leda 35.tif
40mm	leda 40.tif
50mm	leda 50.tif
60mm	leda 60.tif

optimum

focus appears
at $d = 25\text{mm}$!
why?
Eventually need
to test.

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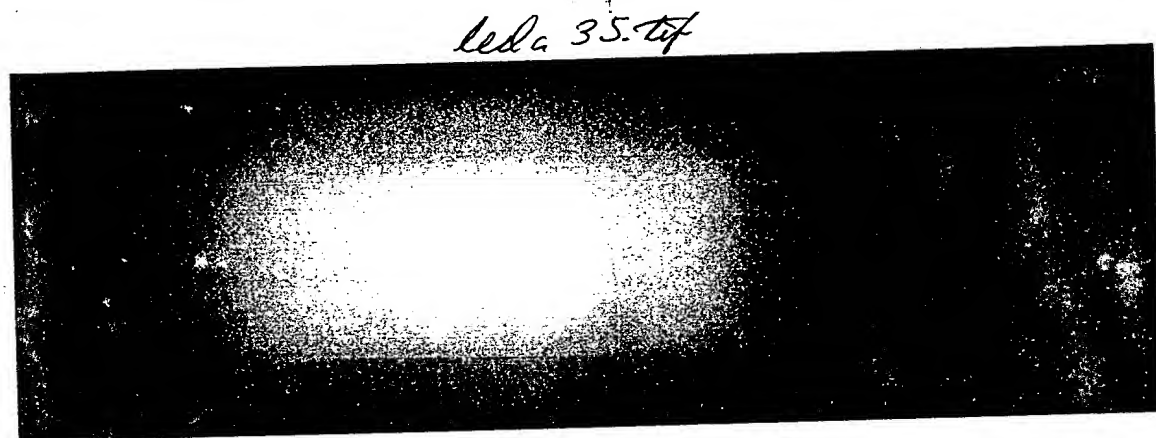
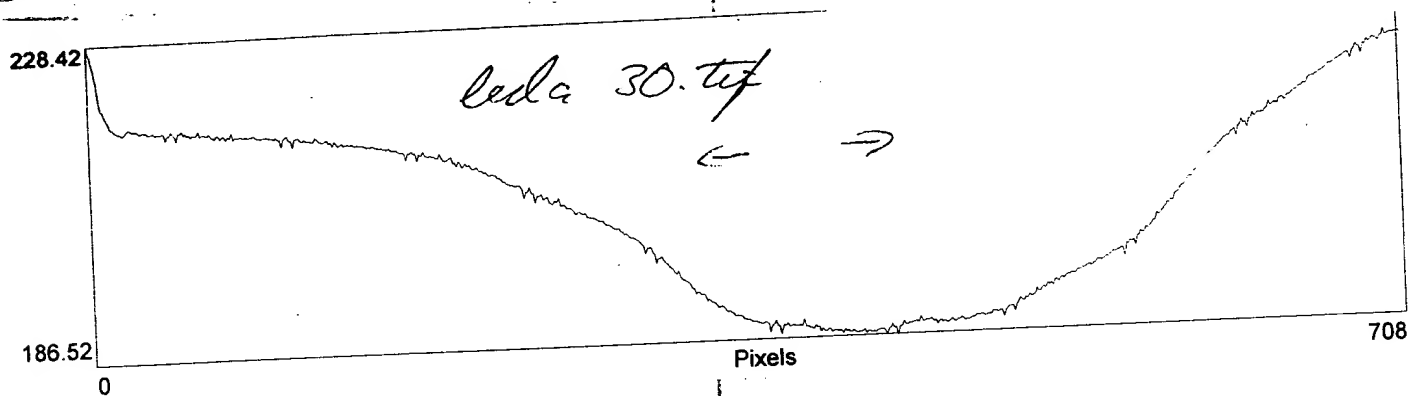
[Signature]

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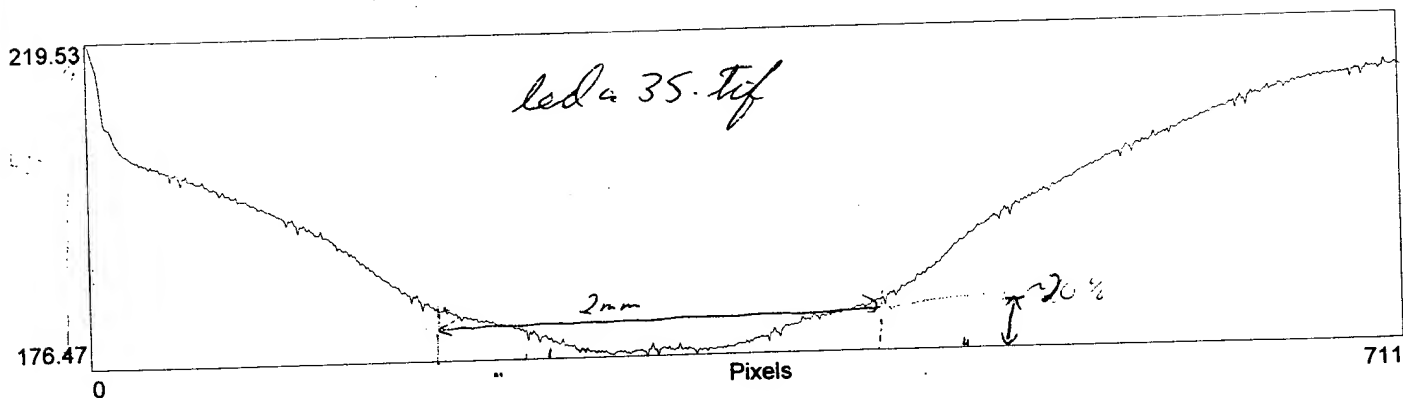
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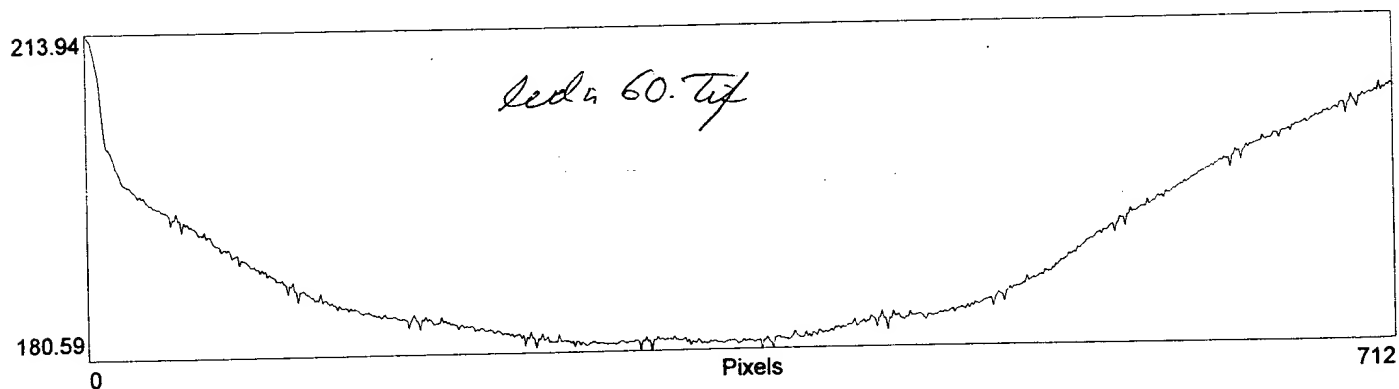
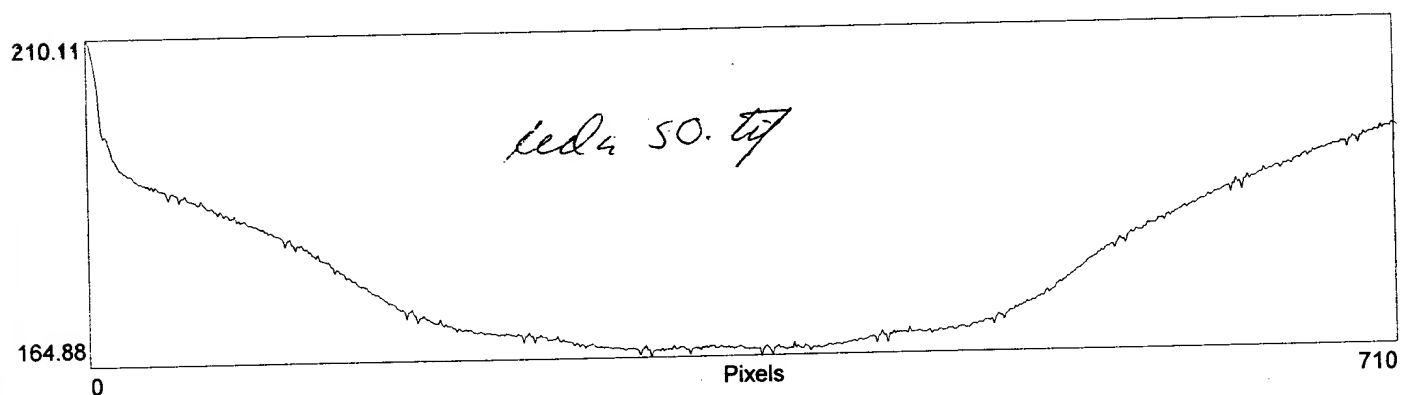
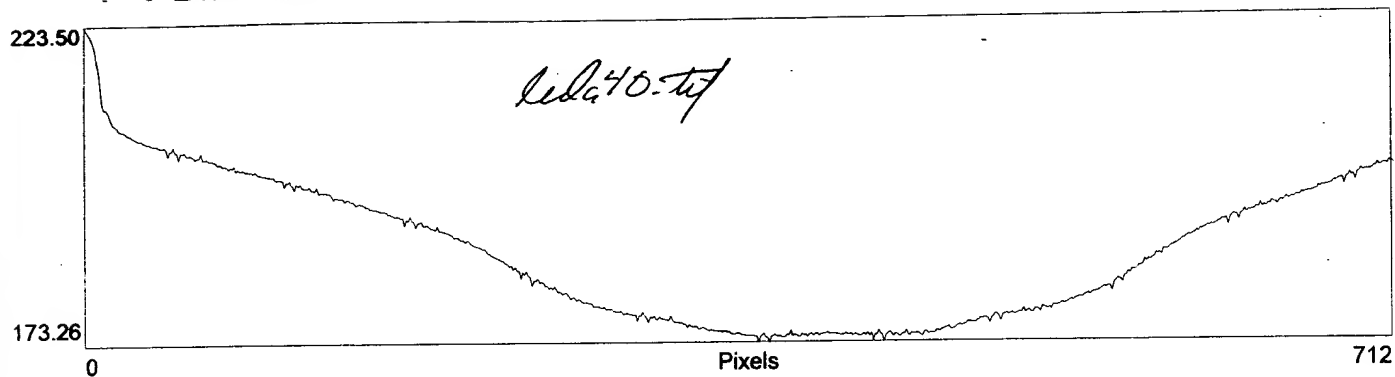
DATE



242 * 716 pixels 6mm wide (716 pixels)



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DATE

11/14/01

WITNESS/TA

DATE

>
 >Tony,
 >
 >I worked last night and got the LED to illuminate the flow cell the way
 >that I want it and imaged beads. It looks great so far. I want to
 test
 >this with a fluorescence filter, but I need to order some 1-inch
 >filters. My "brilliant" idea with the 45-degree filter didn't work
 very
 >well, so I'm going to put a 1-inch filter above the imaging optics. I
 >did a half-baked test with a 3/4" orange plastic filter. It looks very
 >encouraging. I could see beads very well with the power at recommended
 >levels (350 milliamps) and exposure times of only 5 milliseconds,
 better
 >than with our laser.
 >
 >Anyway, I should have some Edmund filters on Monday and we should have
 >the new flow cell materials from the shops then as well. I'll do some
 >more tests on Monday and Tuesday, and by the time you return, I should
 >know what is going to work, and what is not. I might try to image a
 >FITC filled tube as well.
 >
 >If Gus has the flow cell mounts done tomorrow (please check), you might
 >try making a few cells that I can try out next week. Otherwise you
 >might try testing stomaching, if there is an overnight available.
 >
 >We're also going to need Gus to build a better arrangement for holding
 >the LED in a stable way that makes adjustment easy and repeatable.
 >Using a test tube holder is reaching the end of its useful lifetime.
 >
 >Anyway, since things are working, I'm going to leave.
 >

ordered long-pass filters

*Nov 2 email from Franny re
NSF Phase II B mfg.*

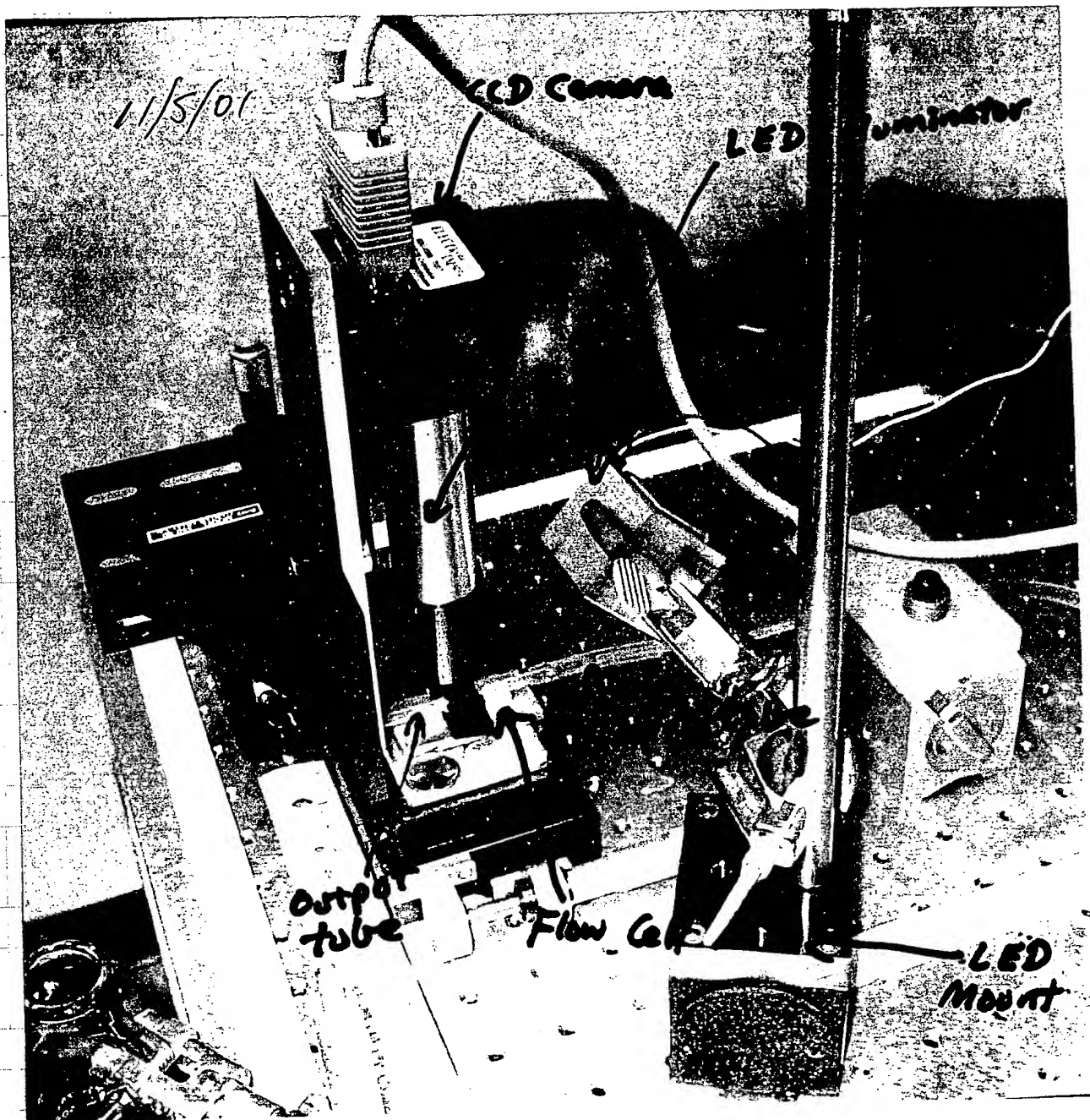
Nov 5

Imaged w/ longpass filters

see setup below

*Received flow-cell mounts
from machine shop.*

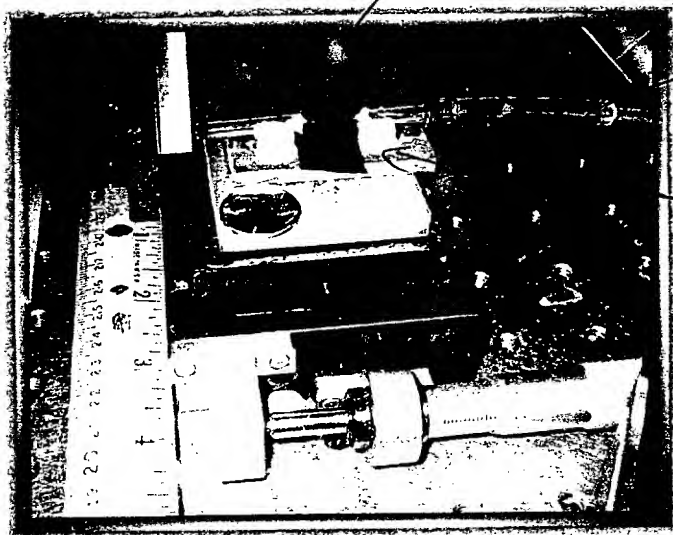
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SIGNATURE <i>RCF</i>	DATE <i>11/14/01</i>	WITNESS/TA	DATE
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11/05/01

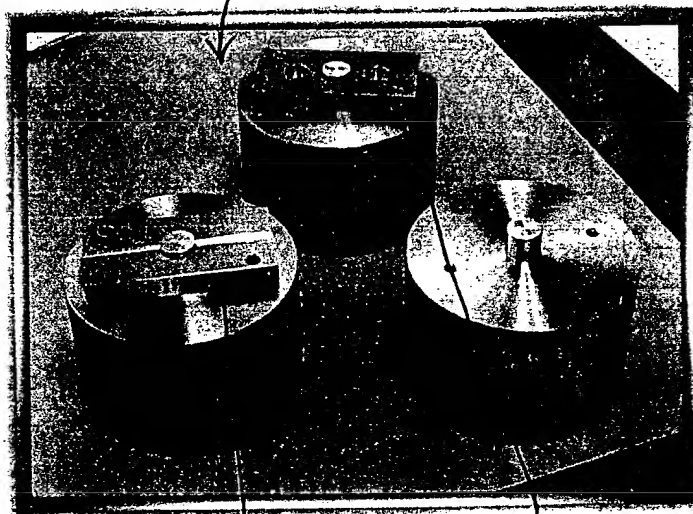
CCD fore-optics



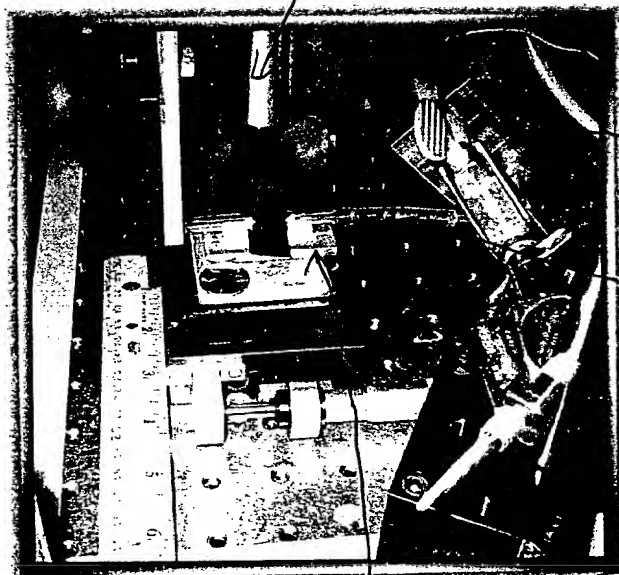
Flow input tube

Flow cell mount

3 Flow Cell Manufacturing Mounts



CCD fore-optics



LED illuminator

Flow Cell Mount

Flow Plumbing

Sub-Flow Cell Structure

Flow cell mount

SIGNATURE

[Handwritten Signature]

DATE

11/11/01

WITNESS/TA

DATE

Nov 5 replied to query from Anna Ost
regarding experimental controls
Lab mtg.

Nov. 6 email from F.C Edman Boeing.

* Draft. press release from Tech link
ordered Blue Dichroic Filter
from Edman 52-531

Nov. 7 performed focus experiment
gave bracket for LED job
to shop


\$ deposit to Bank
Tony built 3 flow cells.

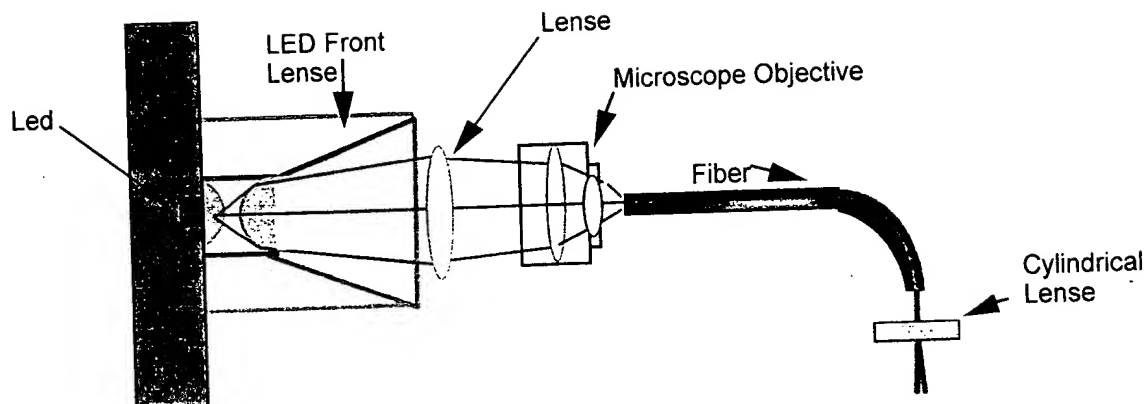
Nov 8

Lab mtg
Experiment w new filter.
WORKS.
Discussed budget w Allison

Nov. 9

Dick sent new optical
design (below)

SIGNATURE 	DATE 8/11/01	WITNESS/TA	DATE
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Paul I'll work on puting this together
on saturday or sunday.

SIGNATURE <i>[Signature]</i>	DATE <i>12/14/01</i>	WITNESS/TA	DATE
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Nov. 12

Spent 1 hour w Amanda
3 hours time sheets + notebook

Had Gus build mode to LED mount.

Nov. 13

called Cooley + Woodward re representation

called Joe re collaboration

worked in lab w Tony looking at
beads w LED illumination

Nov. 14

made calls searching for IP attorney

spent 2 hours in lab w Tony looking
at beads.

called Michael Johnson

Nov. 15

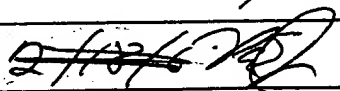
called Joe
ordered filter
lab only

tutted to Gus re spectrometer

spent 2 hours in lab w Tony imaging
beads. TDI works.

Nov. 16

called Colbey + George re getting
prologon.

SIGNATURE 	DATE 2/17/04	WITNESS/TA	DATE
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*talked to Bryan about meeting -
worked in lab w Tony
Nov. 17 shot pictures in lab*

Paul E. Johnson

From: Michael G. Johnson [mjohnson@uark.edu]
Sent: Wednesday, November 14, 2001 11:38 AM
To: Paul E. Johnson
Cc: Mjohnson@Uark. Edu; Rama@Uark. Edu
Subject: RE:

Dear Dr. Paul Johnson:

Do you have a street address for package delivery? If so please send by return email.

The two more recent papers outlining the limitations of our 7G1 monoclonal antibody for L.m. are covered in two pubs by my colleague, Dr. Rama Nannapaneni as follows:

Nannapaneni, R. et al 1998. J. Food Protection 61: 1195-1198 and Nannapaneni et al 1998. Applied Environmental Microbiology 64:3070-3074.

If you would send me a request for some of the 7G1 and mention the reason you want to use it, ala the NSF-SBRI grant proposal and formal title of same, I can then send you a letter of cooperation and some of this antibody for you to test.

Good luck in your application. Sincerely, Mike Johnson, Prof., Food Science Dept., U of Arkansas
-----Original Message-----

From: Paul E. Johnson [mailto:PJohnson@uwyo.edu]
Sent: Wednesday, November 14, 2001 10:34 AM
To: mjohnson@uark.edu
Subject:


Dr. Johnson,

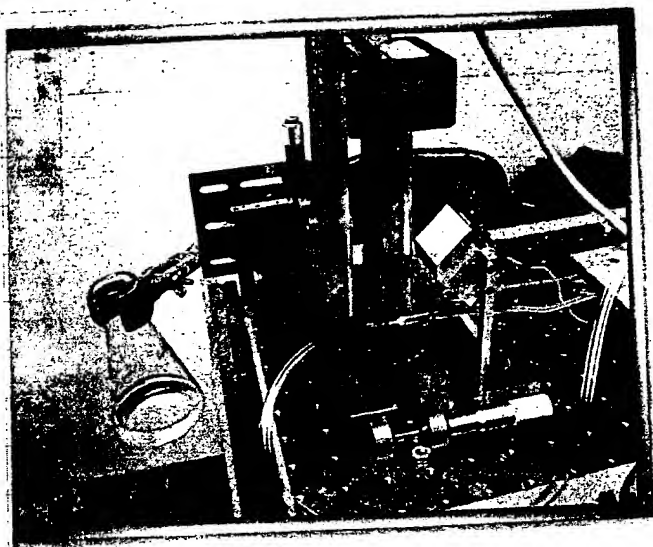
Thank you for taking the time to talk to me this morning. We are looking forward to working with your antibody. My address is:

Dept. of Physics and Astronomy
University of Wyoming
Laramie, WY 82070

My best,

Paul

SIGNATURE 	DATE 2/18/02	WITNESS/TA	DATE
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Nov 17 2001
images

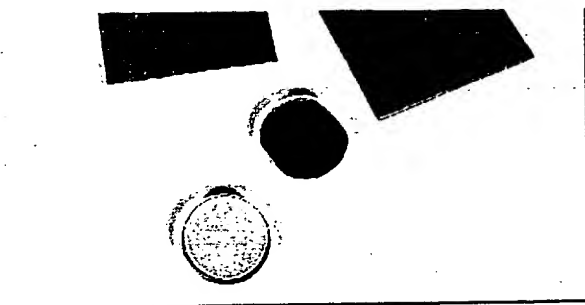
↑
new LED illuminator
illuminating flow cell,
imaged from above



← flow cell
constructed on jig
w RTV (silicone
sement).

SIGNATURE <i>[Signature]</i>	DATE 2/18/02	WITNESS/TA	DATE
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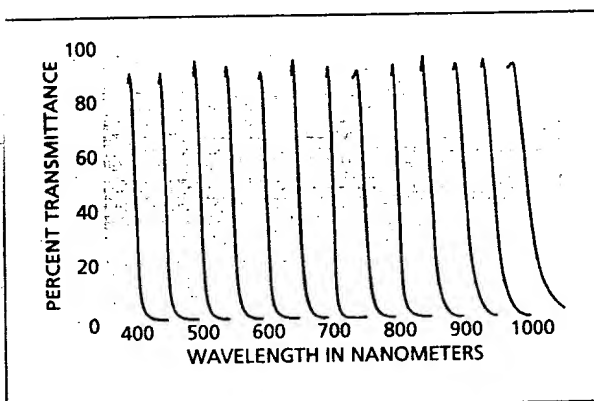
NOTE: INSERT PERIODIC TABLE UNDER COPY SHEET BEFORE WRITING • THE HAYDEN-McNEIL STUDENT LAB NOTEBOOK



Short-Pass Filters

Melles Griot short-pass filters, also called edge filters, have sharp cutoff transitions from reflection to transmission.

- The filters have a steep cutoff edge.
- They are useful for attenuating specific wavelengths.
- They are available in 50-nm increments from 400 nm to 1000 nm.



Typical transmittance curve

SPECIFICATIONS: SHORT-PASS FILTERS

Dimensions:

Diameter: 25.0 +0, -0.15 mm

Square: 50.0 mm × 50.0 mm (±0.25 mm)

Thickness: 1.5 ± 0.5 mm

Clear Aperture:

21 mm for round, 90% of edge dimensions for square

Substrate Material: Fused silica

Wavefront Distortion:

1λ peak to valley per 25-mm area at 632.8 nm over clear aperture

Parallelism: 15 arc seconds maximum

Surface Quality: 80-50 scratch and dig

Operating Temperature: □ 20°C to +200°C

Cutoff Tolerance: □ 10 nm

Angle of Incidence: 0°

Transmission: 85% average over specified wavelength range

Rejection:

99% average, except for 400–450 nm range which is 98% average

Coating:

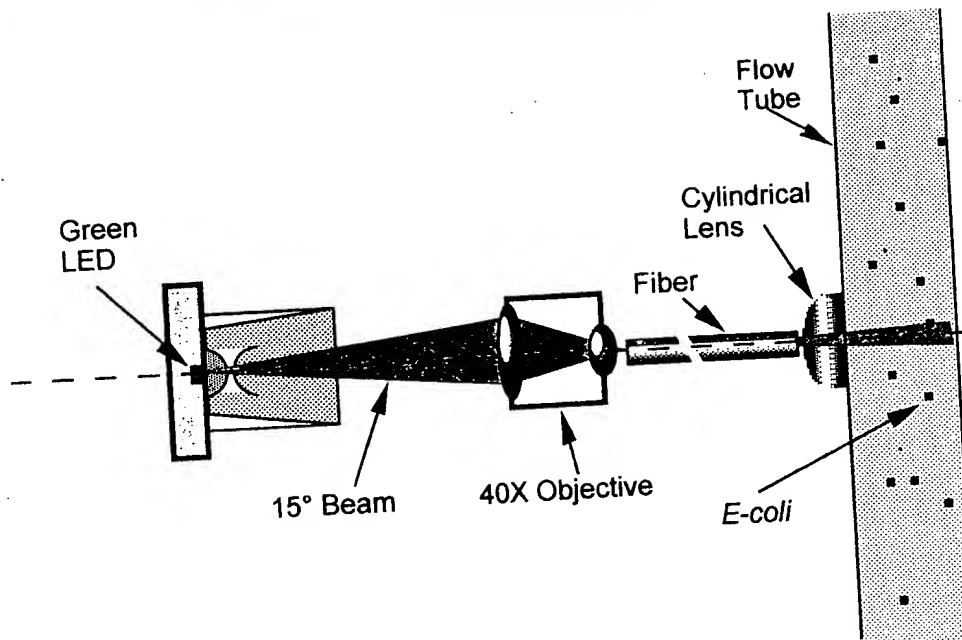
Antireflection coating R ≤ 0.75% over transmission range

Short-Pass Filters

50% Cut-off Wavelength (nm)	Typical Rejection Range (nm)	Typical Transmission Range (nm)	PRODUCT NUMBER	
			25.0 mm Round	50 mm □ Square
400	420–470	285–390	03 SWP 402	03 SWP 602
450	470–530	310–440	03 SWP 404	03 SWP 604
500	525–610	340–485	03 SWP 406	03 SWP 606
550	570–680	375–540	03 SWP 408	03 SWP 608
600	620–790	400–585	03 SWP 410	03 SWP 610
650	675–850	400–640	03 SWP 412	03 SWP 612
			03 SWP 414	03 SWP 614

SIGNATURE <i>[Signature]</i>	DATE 2/18/02	WITNESS/TA	DATE
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Paul E. Johnson

From: Paul E. Johnson
Sent: Tuesday, November 20, 2001 11:01 AM
To: 'Richard W. Shorthill'
Subject: RE: Brightness

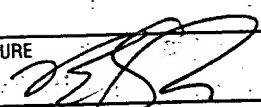
Dick,

Some comments on your drawing:

We have to illuminate the bugs from above (light hitting the broad side of the rectangular tubing), and image them from above. Any technique that places the cylindrical lens on top of the broad side of the tube won't be viable. The illumination has to come in at an angle. Any technique that places the lens on the narrow side of the tube won't work either (the beef extract is opaque over a thickness of 2mm). Secondly, why use a cylindrical lens? The field of view of the CCD has a 6/4 aspect ratio so a square or circular illumination patch is completely satisfactory. Finally, the way this is drawn, you have a 15-degree beam, when in fact we have something like a 110-degree beam. Can you find a microscope objective with a short enough focal length (fast enough f/#) that you actually intercept most of the light emitted by the LED?

Finally the illuminated patch on the optical tube should ideally be about 2mm by 2mm square (perhaps slightly larger) and collimated (or at least not highly divergent over the 100 micron depth of the flow tube).

Paul

SIGNATURE 	DATE 2/18/02	WITNESS/TA	DATE
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7th Oct 21 communicated w Anne Ost
Oct 23
w/ Whipple Grid

From Vick's measurements w scanning objective

$$FOV = 1.15 \text{ mm} \times 1.15 \text{ mm} ?$$

$$10\times \text{ obj.} \rightarrow 0.46 \text{ mm} \times 0.46 \text{ mm}$$

$$20\times \quad .23 \text{ mm} \times .23 \text{ mm}$$

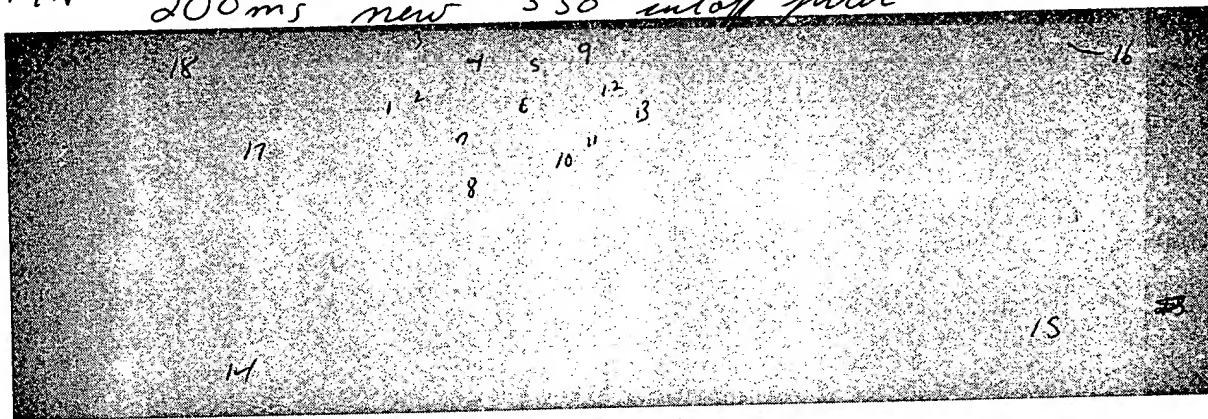
$$\text{normally use} \Rightarrow 40\times \quad .115 \text{ mm} \times .115 \text{ mm}$$

$$100\times \quad .047 \text{ mm} \times .047 \text{ mm}$$

$$40\times \text{ Whipple Grid area } 0.013225 \text{ mm}^2$$

With Tony took Carmine PeakAlign Bead (prober) image. $\checkmark\checkmark$
used Edmund 590nm longpass filter (1")


bead 1. t.f 200ms new 550 intoff filter



some beads stuck together
nearly uniform! surprisingly so!
prelim results $\pm .08$ STDEV
(in excel)

$$14.59$$
$$15.99/2 = .49$$

Ⓢ Sent term sheet to Osman

SIGNATURE 	DATE 2/18/02	WITNESS/TA	DATE
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summary of bead tit photometry

9/20/01
12/23/01

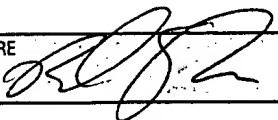
# particles	1	2	3	4
stuck together	0.4	0.85	1.11	1.96
	0.42	0.79		1.83
	0.57	0.71		
	0.59	0.77	1.89 avg	
	0.4	0.84		
		0.92		
	.47 avg $\pm .05$	0.99		
	{ 0.096 }	0.78		
	{ STDEV (in excel)	0.89		
		0.92		

.84 avg $\pm .03$
{ 0.085 }
{ STDEV (in excel) }

Bead

1. .85	9. .84	
2. .40	10. $1.78 \div 2 = .89$	
3. .79	11. $1.84 \div 2 = .92$	} double beads
4. .42	12. 1.96	
5. .40	13. .92	
6. .57	14. .59	
7. .71	15. .99	
8. .77	16. .78	
	17. 1.11	
	18. 1.83	

Photometry
(- background)
using Scion

SIGNATURE 	DATE 2/18/82	WITNESS/TA	DATE
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OMEGA OPTICAL incorporated

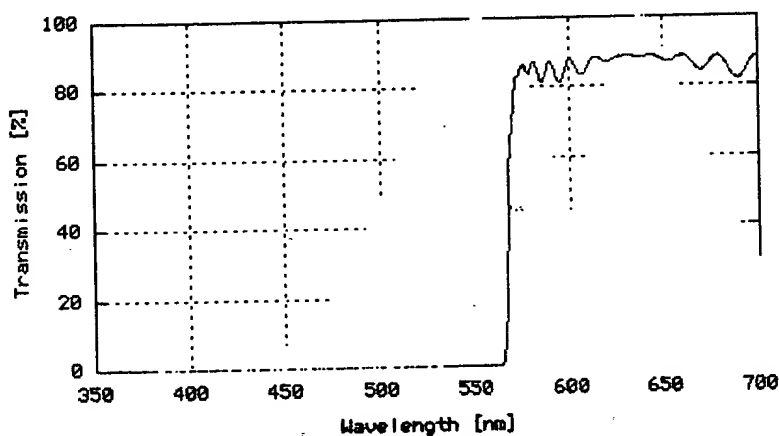
Curv-o-matic

Do not
have this,
but we
should order
something
similar
but about
10 nm
shifted
to red,
for emission
f. filter.

Component **XF3085 (565ALP)**

Fluorophore **Nile Red ***

Compatible: NO



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DATE

2/18/02

WITNESS/TA

DATE

~~11/27/01~~
11/26/01

Set up mtg. with Osman & Humbert.
Forwarded necessary info.

2-hours Worked w Tony in lab starting
TDI.

Set up travel to France.

Met w Gus regarding spectrometer.
Optical design site www.astron.de

11/27/01

Worked w Tony in lab. He
is starting to count carmine beads
do TDI.

Drafted BAA & draft for Leo.
sent.

11/28/01

Worked w Tony in lab setting
up for TDI. need depth of
focus measurement.
Telecon w Dorsey Whitney

11/29/01

Lab meeting.

met w Ryan & Tony in lab.

SIGNATURE



DATE

2/18/02

WITNESS/TA

DATE

*Sample
beads*

*in
beads*

11 29 FD.?, 2.1

11 29 FD. ST. F

200 um down

SIGNATURE

[Signature]

DATE

2/18/01

WITNESS/TA

DATE

11/30/01

email exchange w Anna regarding
contract

Worked w Tony in lab.

Tony ran various experiments:

Paul E. Johnson

From: Tony Deromedi [ajd65@hotmail.com]
Sent: Friday, November 30, 2001 5:43 PM
To: Paul E. Johnson
Subject: beads

Paul - I used the hemacytometer to find the concentration and I got 1.32×10^7 and molecular probes documents showed 1.7×10^7 , so we are as close as you can get. The hemacytometer works way better then the whipple grid, so I guess I'll use it in the future.

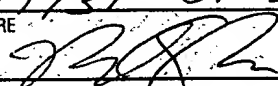
Anyway, I ran the 6.6×10^4 carmine beads through the flow tube varying intensities, flow rate, and exposure time. The results are on a CD that I will leave for you in the physics office. I will also leave the image numbers to which they correspond to with the CD. It seems that the pump rate works the best on 15, at 7.5 no beads were seen even with 350000 and 6 amps. Take a look and see what you think. Give me a call tomorrow at home if you want. 745-4603.

Have a nice evening.

Tony

worked intensively on patent ~~search~~
searches.

11/31 email exchange w Jennifer

SIGNATURE 	DATE 2/18/02	WITNESS/TA	DATE
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Paul E. Johnson

From: Tony Deromedi [ajd65@hotmail.com]
Sent: Sunday, December 02, 2001 11:05 AM
To: Paul E. Johnson
Subject: beads

12/2/01

Hey Paul,

I came in today to try some imaging on the beads. I took a few images with the EDC camera varying the focal distance 70 um up and down. They are saved under d0201(in focus), d0202 (70 up), d0203(70 down). They looked pretty good so like we thought the focusing isn't really our issue. I think we need to figure out if we are getting accurate counts now. I don't know the most efficient way to do this though.

I'm leaving my lab notebook in the lab with the CD that I made on friday.
The images are listed in my lab notebook. I didn't have a key to the physics department so I couldn't leave the results for you on friday.
Sorry about that.

I'll be in tomorrow at 2 so we can talk about it then. Have a nice day.


Tony

12/3/01

received Ryan's photographs on in + out of focus images presentation needs to be reworked.

worked in lab w Tony + Ryan

* met w David Langull re new IP

SIGNATURE 	DATE 2/18/02	WITNESS/TA	DATE
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12/4 and 12/5 \Rightarrow worked on IP \star

Paul E. Johnson

From: Paul E. Johnson
Sent: Wednesday, December 05, 2001 1:54 PM
To: Amanda S. Votaw; 'a_votaw@yahoo.com'

Amanda,


The measurements are starting to go extremely well!

1. We started by taking a stock solution of beads and measuring the concentration with the hemocytometer (Tony doesn't like the Whipple Grid). We get 7.1×10^4 beads/cc.
2. We took a series of still images of beads in the flow cell (having directly measured the field of view and cell depth). We converted these to a concentration. We get 7.2×10^4 beads/cc!
3. Now we flow the same syringe of beads into the flow cell and do TDI. We see about 90% of the beads! Now we're tuning the flow speed and integration time to see if we can improve from 90%.

We've already done multiple runs and we're getting the same answers. I'm getting Ryan to do bead photometry to see how uniformly bright they are in the images. Tony is starting to label K-12 so that we can repeat these measurements with bacteria. It feels like we've crossed the major hurdles on this project.

Paul

12/6 Tony signed IP. \star
Lab only.
Abstract to Alan Abend's meeting.
Ryan signed IP. \star
Amanda reviewing IP

SIGNATURE 	DATE 2/18/02	WITNESS/TA	DATE
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Provisional Patent Application

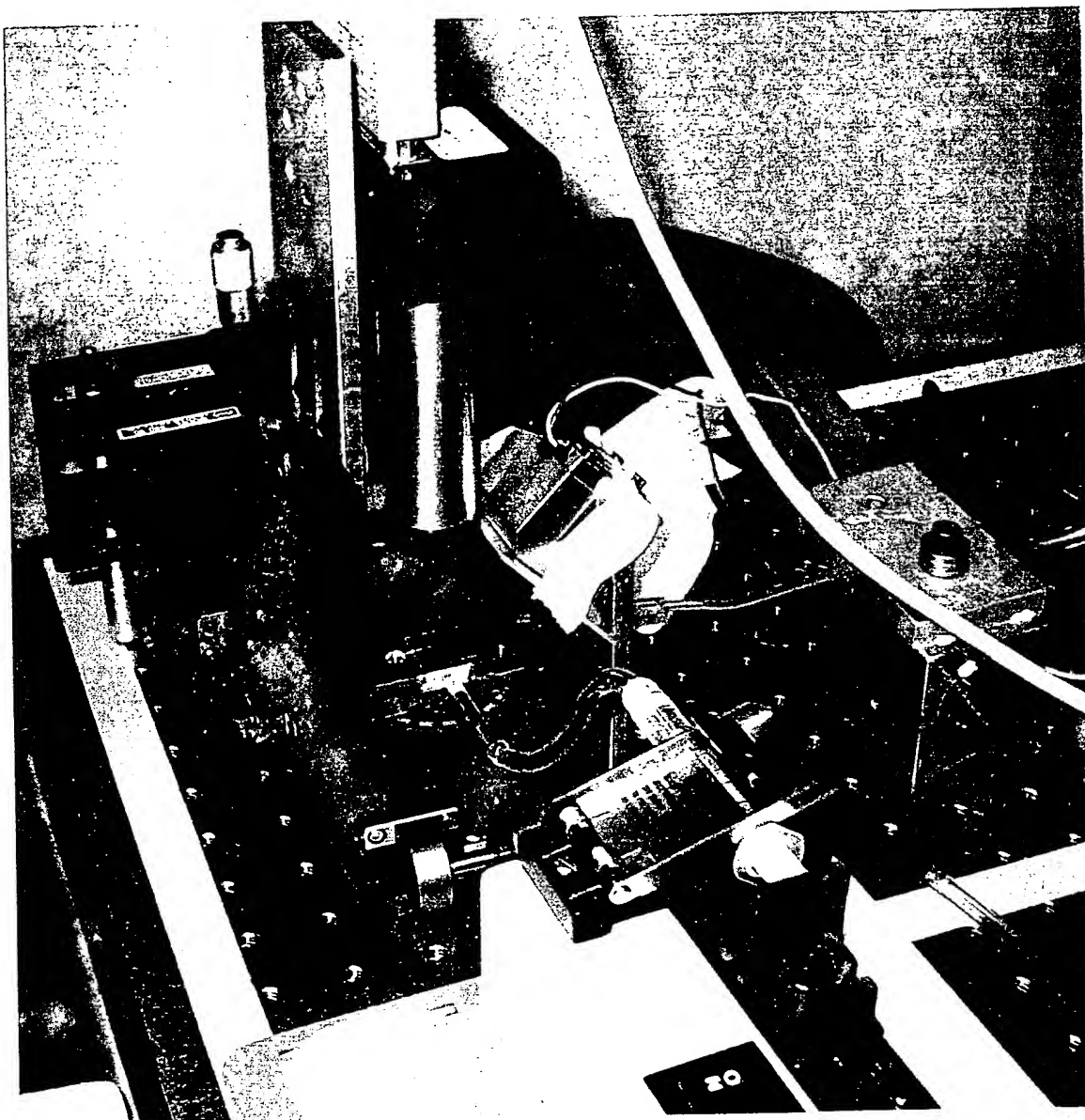


Figure 3. The aluminum flow block shown in figure 2 is shown here mounted on a rotary optical mount. A glass coverslip constrains the liquid flow out of the exit hole in the aluminum block. A CCD camera (black, upper portion of figure) is mounted vertically and connected to fore-optics with an aluminum mounting tube. A fluorescence filter is taped over the fore-optics with electrical tape. Fluorescent beads are pumped into the flow block with a syringe pump. The mouth of the exit hole is illuminated with an LED illuminator (the cube-shaped aluminum block with blue tape on the side).

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12/31 worked on France travel
Do E/Geomet revisor
met w Allison

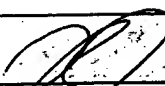
Jan 2 Worked on Geomet MOV
Sent draft below

Draft Minutes of Meeting between SoftRay and BD, December 21, 2001

Present: Noel Warner, Bob Hoffman, Dieter Recktenwald, Jacleen Schmidt, Krissy Manion & Paul Johnson

Recommendations:

1. Marketing of the SoftRay Cytometer must be examined in January for the food processing market in order to enable focused planning for SoftRay R&D, a decision from BD on NSF Phase IIB funding, and to specify more precisely the goals for development of the device, specifically over the next 6 months. Issues to be examined in order to make the device marketable at an acceptable level in the food processing market, including areas outside of ground beef, include:
 - a. required minimum flow rate of the device,
 - b. required sensitivity, specificity, efficiency of detection, and false positive rate, and
 - c. number of simultaneous colors required (probably R-PE and an R-PE conjugate such as CY5)
2. Specifically, to focus R&D of the device, SoftRay should:
 - a. during the next month examine image background issues, e.g. by looking at Quantibrite beads (supplied by RH) in an R-PE background,
 - b. illuminate beef extract with LED and image beads, then bacteria to further test background issue,
 - c. obtain R-PE conjugate from Ken Davies,
 - d. test axial flow vs. TDI to eliminate one
 - e. design and machine two-color device using two image/ two color configuration,
 - f. check into filters (AF) for LED, and
 - g. consider increasing the brightness of LED or use multiple LEDs if necessary.

SIGNATURE 	DATE 1/18/02	WITNESS/TA	DATE
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Dear Dr. Paul Johnson:

Do you have a street address for package delivery? If so please send by return email.

The two more recent papers outlining the limitations of our 7G1 monoclonal antibody for L.m. are covered in two pubs by my colleague, Dr. Rama Nannapaneni as follows:

Nannapaneni, R. et al 1998. J. Food Protection 61: 1195-1198 and Nannapaneni et al 1998. Applied Environmental Microbiology 64:3070-3074.

If you would send me a request for some of the 7G1 and mention the reason you want to use it, ala the NSF-SBRI grant proposal and formal title of same, I can then send you a letter of cooperation and some of this antibody for you to test.

Good luck in your application. Sincerely, Mike Johnson, Prof., Food Science Dept., U of Arkansas

From: Ost, Anna [mailto:Anna.Ost@AirLiquide.com]

Sent: Thursday, January 17, 2002 6:29 AM

To: Paul E. Johnson

Subject: RE: 5.30 Monday 14 January

Dear Paul,

It was nice to see you again! I hope your trip was pleasant both personally and professionally! I am sorry that I could not speak correctly but I think the meeting went well anyhow.

I would like to give you some indications that would shorten the negotiation process so that we can really start to work!

1. Generally, all suggestions you evocated seemed fair to us.
2. The down-payment that you mentionned is ok for us if it could include the first 25 units sold/put in use.

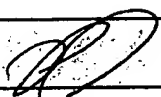
We should also start to discuss the material, for instance could one easily change filters/LEDs for different dyes? What are the dyes that you have already tested?

If we sign the contract say in one month (mid-february), when could you install the equipment do you think?

My best to the whole SoftRay-team

Anna

SIGNATURE



DATE

2/18/02

WITNESS/TA

DATE

EXHIBIT J

Tony Deomedì

Tony Deomedì

1/30 Intensity Experiment / Pump Rate / Exposure time

- WILL PERFORM TOI ON 6.6 x 10⁴ alumina beads
varying LED AMPS (.3 - .6), Pump Rate (15 - 7.5 psi)
Exposure time (700,000 - 350,000)

- 1) 20 IMAGES → .3 AMPS 700,000 ^{15 psi} ^{Flow} 1/30 Image # 1-20
- 2) 20 IMAGES → .6 AMPS 700,000 ^{15 psi} 21-50
- 3) 20 IMAGES → .3 AMPS 700,000 ^{7.5 psi} 67-90
- 4) 20 IMAGES → .6 AMPS 700,000 ^{7.5 psi} ^{Flow} 1/30 1-20
- 5) 20 IMAGES → .3 AMPS 350,000 ^{15 psi} ^{EN30} 1-25
- 6) 20 IMAGES → .6 AMPS 350,000 ^{15 psi} 25-60
- 7) 20 IMAGES → .3 AMPS 350,000 ^{7.5 psi} 75-100
- 8) 20 IMAGES → .6 AMPS 350,000 ^{7.5 psi} 100-150

→ Beads were stirred for 30 minutes then sonicated for 4 minutes then vortexed for 1 min

20 IMAGES .3 AMPS 700,000 ^{25 PSI} 1-30
20 IMAGES .6 AMPS 700,000 ^{25 PSI} 30-60

^{EN30}
.3 AMPS 700,000 ^{25 PSI} 1-30
.6 AMPS 700,000 ^{25 PSI} 30-60

7.5 PSI → NO IMAGES - Maybe try stretching them or something

SIGNATURE	DATE	WITNESS	DATE
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Tony Devereaux

12/02/01: Stirred 6.6×10^4 Carmine beads for 30 minutes,
sonicated for 4 min, vortexed 1 min.

Ran small amount through flow cell.

Saved Images to EDC software:

00201 \rightarrow In focus

00202 \rightarrow 70 μ m up

00203 \rightarrow 70 μ m down

\rightarrow Focus really isn't the issue.

12/03/01: Measure Beads with hemacytometer \rightarrow

\rightarrow 10 still frames d 3s (1-10) (.6 amps)

\rightarrow 125 images (.6 amps) (700,000) (15 PSI)
d03t (1-125)

\rightarrow 10 still frames d 3s (11-20) (.6 amps)

\rightarrow 125 images (.6 amps) (700,000) (15 PSI)

1 11
2 11
3 9
4 2
5 9

6 14
7 7
8 12
9 7
10 7

8% = 8.9×10^4

8.9×10^4

SIGNATURE

DATE

WITNESS/TA

DATE

104 Journal

12/30/01: VAMP Integration time from $\frac{2.1 \text{ million}}{2.52 \text{ million}} \times .20 = 420000$
 1.86 million
 1.44 million
 1.02 million
 2.94 million
 3.36 million

@ .6 AMMS & 5 PSI.

2.1	dosa	1-40	- Nothing
2.52	dosa	1-20	- Nothing
2.94	dosc	1-20	- Nothing
3.36	dosd	1-20	- Nothing
1.86	dose	1-10	- Nothing
1.44	dosf	1-10	- Nothing
1.02	dosg	1-10	- Nothing
4.00	dosh	1-10	nothing
1.15	dosi	1-10	nothing
1.15	dosi		
1.20	dost	1-10	nothing

12/30/01: Lab meeting

- Try to put FITC Ab on K12.
- Followed protocol for Ab labelled
- Can't see anything

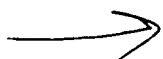
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NOTE: INSERT PERIODIC TABLE UNDER COPY SHEET BEFORE WRITING • THE HAYDEN-McNEIL STUDENT LAB NOTEBOOK

12/19/11 Setup New Flow cell.

Save all Still Images UNDER

.6 AMPS 3 PSI



1214 NC → no coverslip

1214 NC 2 →

1214 CS 1 →

1214 CS 2 →

Took 100 Images under IDC
@ 3 PSI & .6 AMPS

File name

1214 C 1 - 100

— coverslip → Look good;

1214 N 1 - 100

→ No coverslip →

→ Painted well with Black Paint
took ten more images.

maybe less background scattered light.

SIGNATURE	DATE	WITNESS/TA	DATE
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Tom Dwyer

1/24/92: General Lab setup + spike to Amersham about R phosphor -
Stirred 10^6 c.c.m. beam for 30 min
Sonicated for 5 min
Vortexed 1 min

- WILL TRY AGAIN TO CHANGE GAIN + BIAS on
the software to see if images are better. using FF

20 images of each @ 300 exposure time + 15 PSI

GAIN 50
Bias 100 0124a

GAIN 50
Bias 125 0124b

GAIN 50
Bias 150 0124c

⑥ 50 0124d
⑧ 175

GAIN 50 0124e
Bias 200

GAIN ~~200~~ 150 0124f
Bias 200

GAIN 150 0124j
Bias 100

GAIN 150 0124g
Bias 175

GAIN 150 0124h
Bias 150

GAIN 150 0124i
Bias 125

All SEEM ~~DIFF~~ SIMILAR

All the same

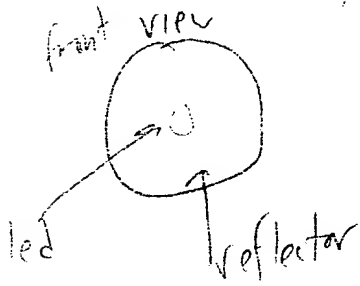
SIGNATURE	DATE	WITNESS/TA	DATE
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EXHIBIT K

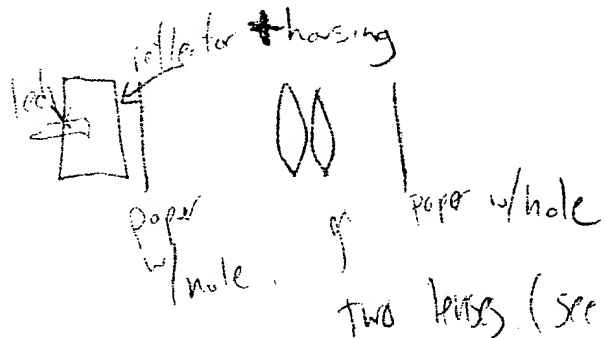
Nick Deskevich

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	33
NAME	LOCKER/DESK NO.	COURSE & SECTION NO.	
NICK DESKEVICH			

LEDs finally came
they project much like a flashlight
try to ~~focus~~ focus down to a point.



put a piece of black paper over the
led. Paper has a hole in it.
This reduces amount of light spreading out.



used two lenses to
focus beam. Everything
held together w/ wooden
sticks, black paper and
tape.

This setup focuses
down to a spot.
Only works at a
fixed distance unlike
a laser. This can
be changed by moving
the lenses. A laser
line generator in
front produces a ~~focused~~
focused line about
3mm long.

Valid
5-8-02

SIGNATURE	DATE	WITNESS/TA	DATE

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	34
NAME NICK DESKEVICH		COURSE & SECTION NO.	

The green LED focused down to a line seems like it will light up the right size and shape part of the flav cell. My line is slightly uneven but with more careful construction i.e. not sticks, paper, and tape, a better line could be produced. I will hold off making something better until I can get a better filter. The filters I have available work for the green laser (532?) but they pass through a bunch of LED light. I also don't know if the LED will be bright enough to be effective. A lot of light is lost in my blocking and focusing set up. I tried 7, 9, and 24 LED arrays but when focusing I get individual dots (7, 9 or 24) when I block them off, I only get the light of one LED from the array. A single super bright LED like the one from Lumileds seems to be the best way to go.

COPY

Valid
3-8-07

SIGNATURE	DATE	WITNESS/TA	DATE
-----------	------	------------	------

EXHIBIT L

September 21, 2000

LED types:

(532 nm) Green Small → NSPG300A
Large → NSPG500S

(450-465) nm Bluish-Green Large → NSPE590S

	OPTICAL POWER OUTPUT (mW)	DC FORWARD VOLTAGE (V)		DC REVERSE CURRENT (μA)	POWER DISSIPATION (mW)	LUMINOUS INTENS. (cd)	DIR *
		TYP	MAX				
①	4	3.5	4.0	50.0	120	6.80	15°
②	4	3.5	4.0	50.0	120	18.00	15°
③	4-6	3.5-3.6	4.0	50.0	120	?	

* Directivity 2θ theta; $\frac{1}{2}$

For All:

DC FORWARD CURRENT = 30 mA ←

PULSE FORWARD CURRENT = 100 mA

DC REVERSE VOLTAGE = 5V

OPERATING TEMP = -30 ~ +85

STORAGE TEMP = -40 ~ +100

* Run at 20 mA, 25 mA, 30 mA

For short time → MAX

VKB
11-06-00

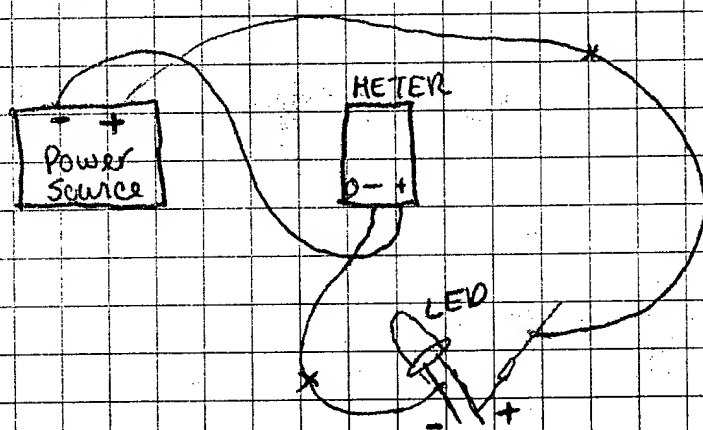
September 28, 2000

- Biohazard Training
- Test LED's (Find 25 mA)
- New flatpack LED's in → NSPGF505

October 5, 2000

Large Green (NSPG5005) (w/o brass coding)

LED #1 → Visible Light @ .03 mA



Current (mA)	Power (mW)	→ Measured fr. Laser Check (right next to LED)
0.03	—	
5.03	0.79	
10.02	1.35	
15.01	1.84	
20.06	2.18	
25.04	2.77	
26.57	2.82	

✓ KLB
11-06-00

Large Bluish-Green LED (NSPE5905) (w/o Brass Casing)

LED #2

(w/o resistor)

Current (mA)	Power (mW)
5.04 ± 0.05	0.33 ± 0.02
9.97	0.61
15.08	0.87
20.16	1.13
24.99	1.34
30.10	1.52

Small Green (NSPG300A) (w/o Brass Casing)

LED #3

(w/o resistor)

Current (mA)	Power (mW)
5.02	0.41
10.02	0.67
15.02	0.87
20.13	1.06
25.02	1.22
30.05	1.34

October 11, 2000

LED # 4,1 → Large Green (NSPG 500 S)
 LED # 5,2 → Large Bluish-Green (NSPE 590 S)
 LED # 6,3 → Small Green (NSPG 300 A)

LED # 2 (w/resistor) (w/o brass coding)

Current (mA)	Power (mW)
4.65	0.32
9.67	0.64
15.00	0.82
19.46	1.06
25.60	1.34
30.78	1.89

LED # 5 (w/resistor) (w/o brass coding)

Current (mA)	Power (mW)
4.89	0.35
10.54	0.62
14.03	0.98
20.18	1.22
24.63	1.50
29.92	1.74

LED # 3 (w/resistor) (w/o coding)

Current (mA)	Power (mW)
5.50	0.46
9.58	0.68
14.19	0.88
19.35	0.99
24.75	1.31
30.03	1.45

LED # 6 (w/resistor) (w/o coding)

Current (mA)	Power (mW)
5.74	0.43
9.68	0.58
14.56	0.80
19.80	1.01
24.61	0.98
29.45	1.29

✓KAB
11-06-00

October 12, 2000

05

LED #4

(w/resistor) (w/o rasing)

All Power readings taken

w/ing

Laser Check

Current (mA)	Power (mW)
4.86	0.89
10.20	1.86
15.03	2.44
20.10	2.82
24.71	3.37
≈ 29.00	3.74

LED #7

Large Green (NSPG 5005)

(w/resistor) (w/o rasing)

Current (mA)	Power (mW)
22.22	0.89
5.05	0.98
10.21	1.78
14.57	2.22
20.03	2.82
24.40	3.15
≈ 29.97	3.85

LED #8

Large Green (NSPG 5005)

(w/resistor) (w/o rasing)

Current (mA)	Power (mW)
5.61	1.22
9.40	1.93
15.09	2.61
20.38	3.17
24.85	3.56
30.13	4.04

✓ KLB
11-06-00
Julie Kellogg

October 18, 2000

LED #9

Large Green (NSPG500S)
(w/resistor) (w/o reading)

Current (mA)	Power (mW)
4.34	0.97
9.67	1.73
14.90	2.39
19.71	3.13
24.80	3.45
30.14	4.37

LED #11

Large Green (NSPG500S)
(w/resistor) (w/o reading)

Current (mA)	Power (mW)
5.10	1.07
10.80	2.07
15.60	2.62
19.60	3.12
25.56	3.65
30.28	4.15

LED #12

Large Green (NSPG500S)
(w/resistor) (w/o reading)

Current (mA)	Power (mW)
5.90	1.35
11.38	2.24
14.58	2.53
19.34	3.34
24.60	3.87
30.60	4.23

LED # 13

Large Green (NSPG500S)
(w/ resistor) (w/o resistor)

Current (mA)	Power (mW)
4.42	1.06
9.40	1.91
15.28	2.54
20.85	3.17
25.36	3.70
30.25 29.25	3.56 4.23

LED # 14

Large Green (NSPG500S)
(w/ resistor) (w/o resistor)

Current (mA)	Power (mW)
5.09	1.15
10.94	2.16
14.86	2.75
19.85	3.22
24.50	3.89
29.82	4.18

LED # 15

~~Large Green (NSPG500S)~~ Large Bluish-Green (NSPE590S)
~~(w/ resistor) (w/o resistor)~~

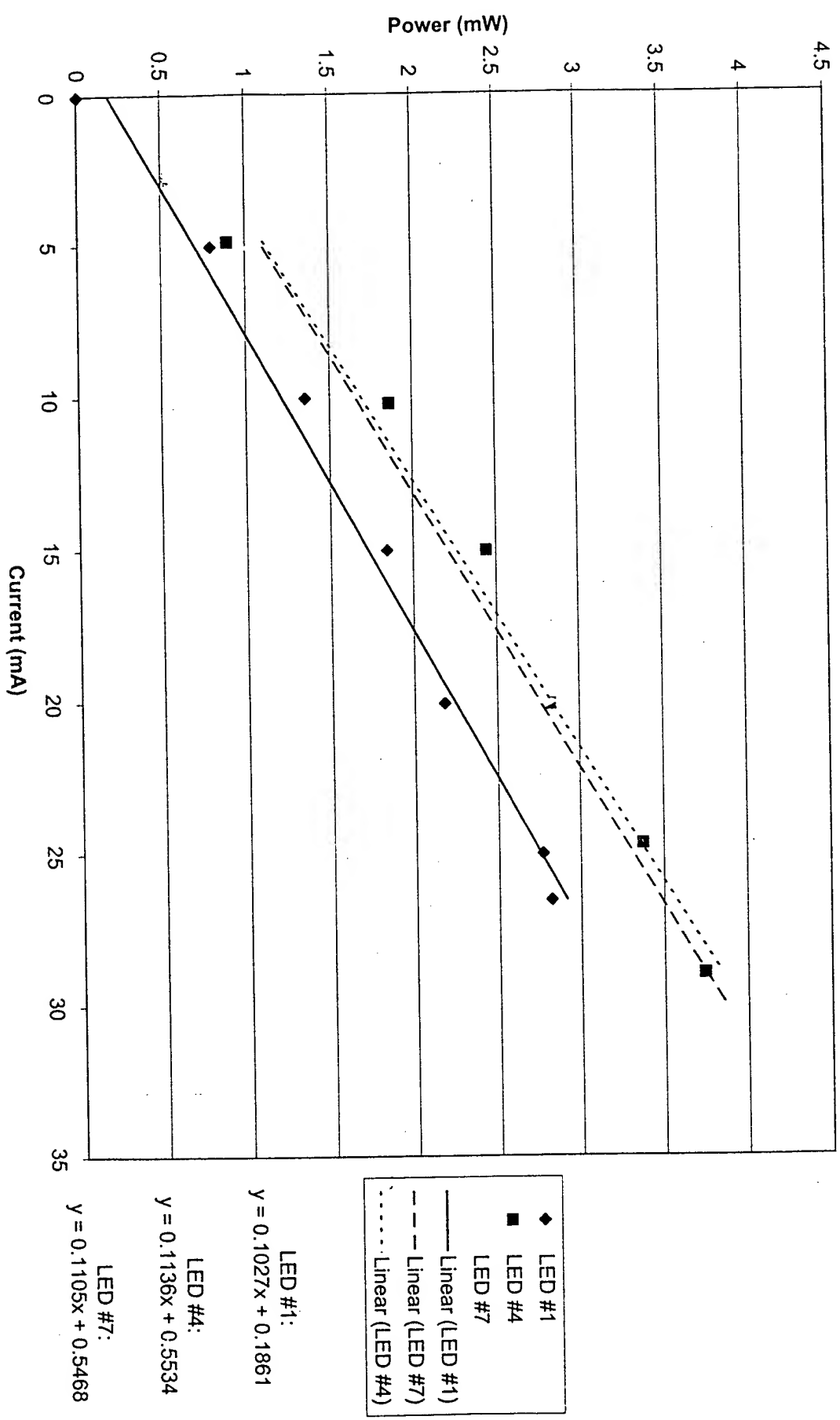
Current (mA)	Power (mW)
4.99	0.15
9.74	0.24
14.58	0.37
19.79	0.46
24.60	0.54
30.30 29.38	0.55 0.61

JKB
11-06-00* At 30mA, we see a drop
(steady) in Power.Julio
Kellogg

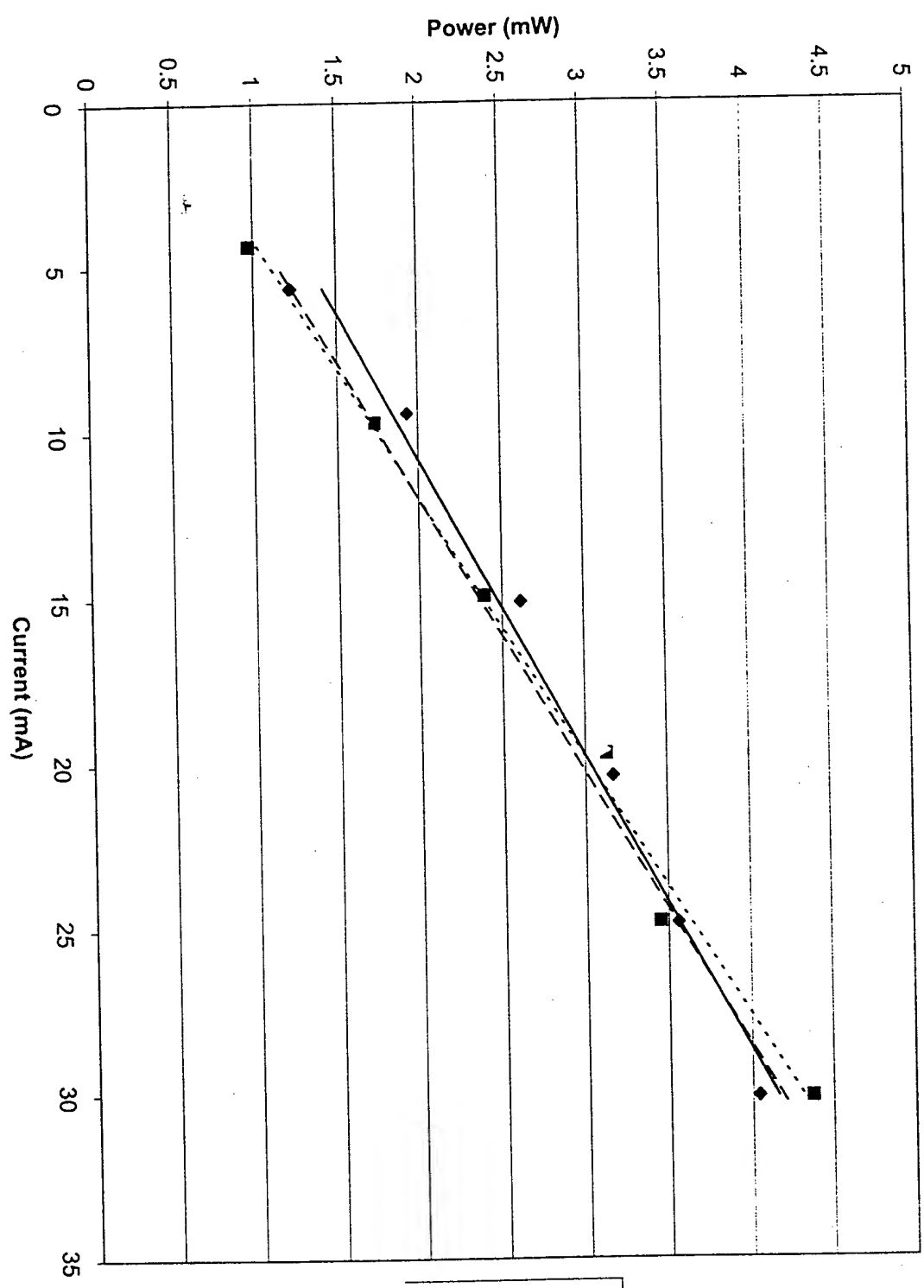
10/18/00

✓ KTB
11-02-00

LED Power Output



LED Power Outputs



◆ LED #8
■ LED #9
▲ LED #11
--- Linear (LED #11)
— Linear (LED #8)
-.-.- Linear (LED #9)

LED #8:
 $y = 0.1119x + 0.7884$

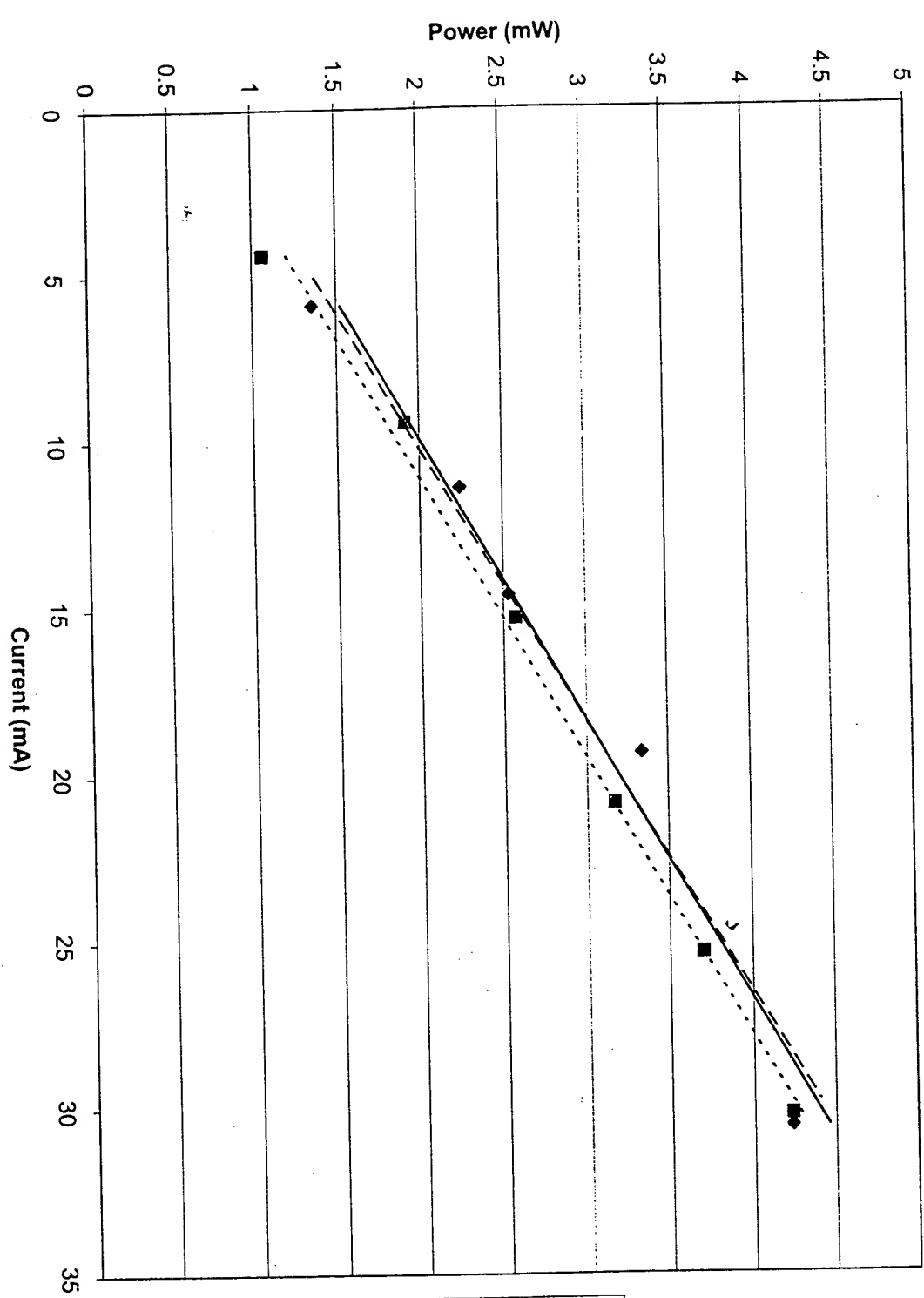
LED #9:
 $y = 0.1278x + 0.4667$

LED #11:
 $y = 0.1209x + 0.548$

10/11/00

✓ KTB
11/06/00

LED Power Output



- ◆ LED #12
 - LED #13
 - ✕ LED #14
 - Linear (LED #12)
 - - - Linear (LED #13)
 - - - Linear (LED #14)
- LED #12:
 $y = 0.1188x + 0.8202$
- LED #13:
 $y = 0.1195x + 0.6701$
- LED #14:
 $y = 0.1229x + 0.74$

October 19, 2000Large Green LEDsRanking according to fitted slope

LED#1

9

LED#4

6

LED#7

8

LED#8

7

LED#9

1

LED#11

3

LED#12

5

LED#13

4

LED#14

2

Large Bluish-Green LEDs

LED#2

LED#5

October 19, 2000

To Do: Fit one (not best) LED into brass casing w/ epoxy
(grind off optics & sand down)

Take 3 or so runs @ range of currents to compare.

See if power drops off @ 30 mA.

→ Test Blue & small Green LED's

→ Make smooth plots of graphs

October 24/25, 2000

USE LED #1 → Remove optics

• Cut off and sanded optics w/ Dremmel Tool

• Epoxy LED #1 into optics casing

Measure Power output from casing

RUN #1 LED #1 in casing

Current (mA)	Power (mW)
5.08	0.43
10.37	0.77
15.71	1.07
19.67	1.22
25.42	1.43
28.53	1.62

Did see a decrease
in power @ 30 mA

RUN #2

Current (mA)	Power (mW)
4.59	0.40
11.01	0.82
16.47	1.11
20.00	1.26
24.38	1.46
29.50	1.62

Did see ↓ in P
@ 30 mA

VKJB
11-06-00

RUN #3

<u>Current (mA)</u>	<u>Power (mW)</u>
20.00	1.00
6.25	0.51
9.89	0.74
14.45	0.93
19.57	1.25
25.06	1.49
29.90	1.66

Die see drop in P
@ 30 mA

November 1, 2000 / Nov. 6, 2000

11

Measure inner diameter of coating:

inner
coating
OD $\approx 0.355''$
 $= 9.017$

outer
coating $= 0.395''$
ID $= 10.033 \text{ mm}$

USE LED #12 \rightarrow Remove Optics

- Cut off & sand optics w/ Diamond Tool
- Epoxy #12 into coating

Measure Power Output

Run #1 LED #12 in Coating

Current (mA)	Power (mW)
4.66	0.43
10.58	0.82
14.83	1.04
20.46	1.28

saw decrease
in Power
@ 20mA

Run #2

Current (mA)	Power (mW)
5.96	0.51
10.06	0.78
15.46	1.08
20.24	1.28

Run #3

Current (mA)	Power (mW)
4.82	0.44
11.35	0.85
15.53	1.07
20.55	1.30

Julio
Kelllogg

AKB
12-4-00

November 6, 2003

12

TEST Flat-pack LED's

(w/resistor)
(w/o cooling)

LED's # 15 - 24

LED # 15

Current (mA)	Power (mW)
5.16	0.36
10.09	0.59
15.37	0.83
20.00	1.02

LED # 19

Current (mA)	Power (mW)
5.57	0.33
9.80	0.49
14.65	0.65
20.30	0.93

LED # 16

Current (mA)	Power (mW)
5.34	0.34
10.06	0.55
15.26	0.76
19.47	0.93

LED # 20

Current (mA)	Power (mW)
5.07	0.34
10.95	0.64
14.66	0.93
20.40	1.06

LED # 17

Current (mA)	Power (mW)
4.58	0.38
9.95	0.71
15.57	0.98
20.25	1.21

LED # 21

Current (mA)	Power (mW)
5.96	0.41
10.42	0.64
15.80	0.91
20.40	1.04

LED # 18

Current (mA)	Power (mW)
5.21	0.41
10.40	0.71
15.30	1.03
20.00	1.13

LED # 22

Current (mA)	Power (mW)
4.88	0.34
10.70	0.64
14.70	0.93
20.88	1.00

✓ KB
12-4-00

LED #23

Current (mA)	Power (mW)
5.22	0.43
10.72	0.73
15.26	0.98
20.15	1.12

LED #24

Current (mA)	Power (mW)
4.90	0.39
10.00	0.60
15.28	0.87
19.90	1.04

11/20/00

Blue LEDs (large NSPE5905)

(LED'S #25 - #27)

LED #25

Current (mA)	Power (mW)
4.63	0.32
10.66	0.65
15.43	0.92
19.50	1.07

decrease in
luminosity
@ 20 mA

LED #26

Current (mA)	Power (mW)
4.93	0.30
9.93	0.54
15.24	0.82
19.80	1.07

decrease in
Power/luminosity
@ 20 mA

LED #27

Current (mA)	Power (mW)
4.58	0.31
9.90	0.59
16.78	0.89
19.96	1.07

decrease in
Power @ 20 mA
after ~ 5 sec.

✓ KB
12-4-00

Julie Keller
11/20/00

11/29/00

Small Green LEDs (NSPG300A)

(LED # 28 - # 34)

LED # 28

Current (mA)	Power (mW)
--------------	------------

4.76	0.38
------	------

10.28	0.61
-------	------

15.93	0.81
-------	------

20.10	0.87
-------	------

decrease in
Power @
20mA after
a few
seconds

LED # 29 (LED's # 25 - # 27)

Current (mA)	Power (mW)
--------------	------------

4.37	0.30
------	------

10.35	0.62
-------	------

14.97	0.81
-------	------

20.03	0.99
-------	------

decrease
in Power @
20mA after
a few seconds

LED # 30

Current (mA)	Power (mW)
--------------	------------

4.76	0.38
-----------------	-----------------

5.29	0.45
------	------

10.81	0.67
-------	------

14.96	0.82
-------	------

20.24	0.93
-------	------

decrease in
Power @ 20mA
after a few seconds

LED # 31

Current (mA)	Power (mW)
--------------	------------

4.62	0.34
------	------

9.64	0.58
------	------

15.03	0.77
-------	------

20.03	0.94
-------	------

decrease in
Power @ 20mA
after a few
seconds

LED #32

Current (mA)	Power (mW)
5.10	0.36
10.51	0.58
15.88	0.81
20.33	0.92

decrease in
Power after
a few seconds
@ 20 mA

LED #33

Current (mA)	Power (mW)
5.28	0.40
11.65	0.76
15.42	0.95
20.51	1.09

decrease in Power
@ 20 mA
after a few
seconds.

LED #34

Current (mA)	Power (mW)
5.04	0.45
10.46	0.75
15.46	0.99
20.57	1.10

decrease in Power
@ 20 mA after
a few
seconds.

November 29, 2000

New Large Green LEDs (NSPG5005)

(LED's #36 - #45)

Tested only every $\approx 2\text{mA}$ to 10mA to prevent fatigue.LED #36

Current (mA)	Power (mW)
1.96	0.52
3.77	0.85
5.93	1.26
7.83	1.37
10.01	1.70

LED #37

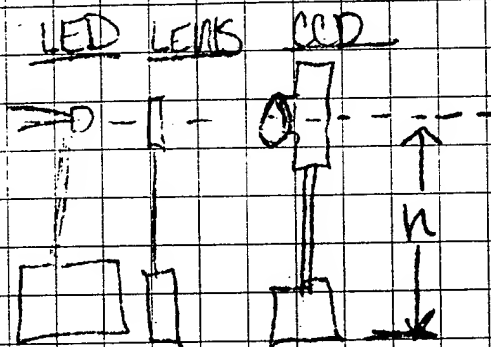
Current (mA)	Power (mW)
1.87	0.43
3.89	0.95
6.24	1.25
8.07	1.53
10.16	1.74

LED #38

Current (mA)	Power (mW)
1.91	0.48
3.96	1.00
5.99	1.34
7.98	1.61
10.10	1.83

January 18, 2001

Setup for testing cylindrical lenses:



Take pics
of illumination
Change

- dist. of LED fr. Lens
- dist of camera fr. LENS
- placing of LENS

January 23, 2001

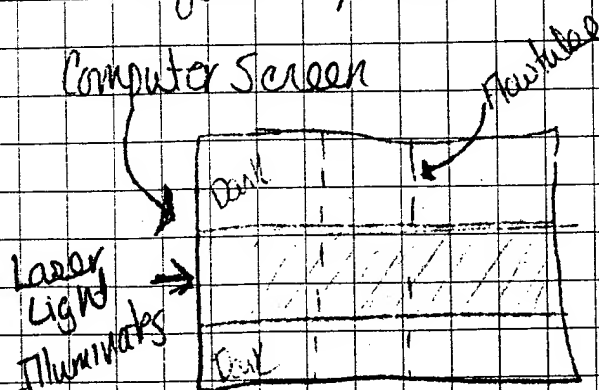
Align instruments \rightarrow LED, Optics, CCD

- Waiting for Power Source

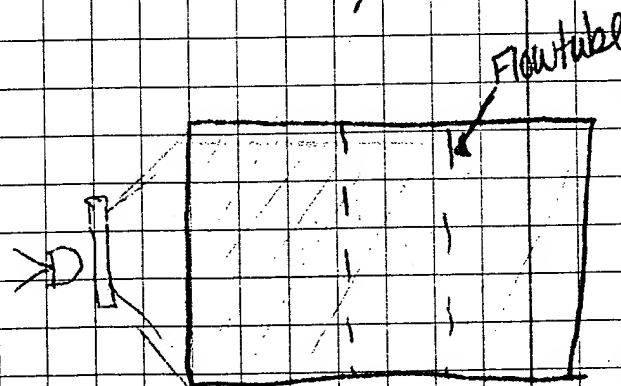
$h \rightarrow$ Up 21 1/2 cm aligned

January 25, 2001

Before (w/ Laser):



Need w/ LED:



VKB
2-03-01

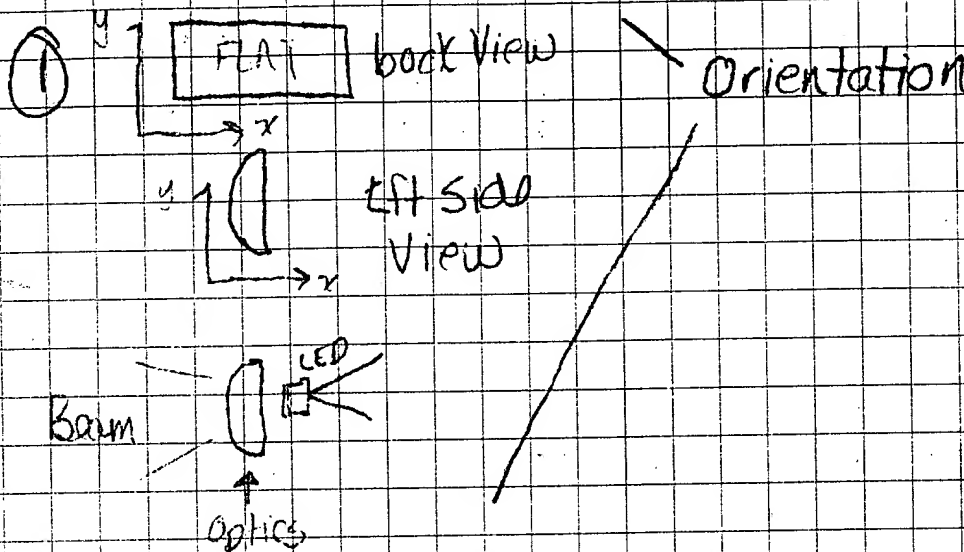
* Illuminate entire flowtube

January 30, 2001

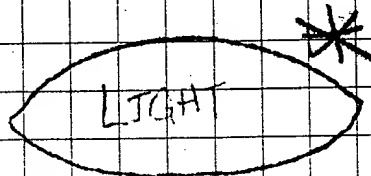
Pictures w/ CCD camera proved futile
w/ this setup \rightarrow Either No Light or
All Light.

Shined LED against black background
to see shape of beam

(All done w/
lg Green LED #12
w/ sanded
optics in brass
casing.)



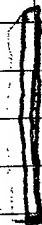
Beam Shape :



(2) Orientation :



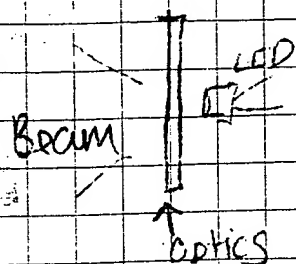
Back View



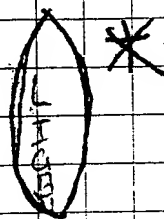
Side View

JK

VKB
2-02-01

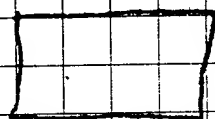


BEAM SHAPE:

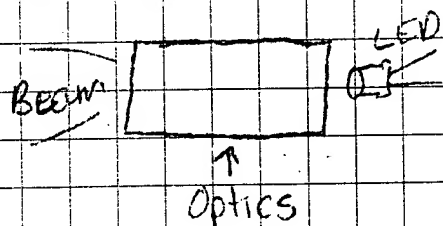


③ Orientation

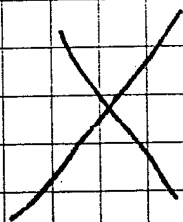
Back View



Side View



BEAM SHAPE:



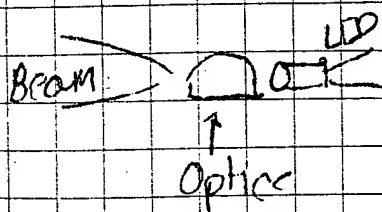
NOT GOOD

④ Orientation

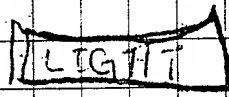
Back View



Side View



BEAM SHAPE:

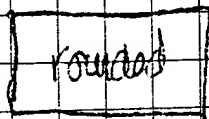


with
lots
of extra
Background
light.

⑤ *

Orientation

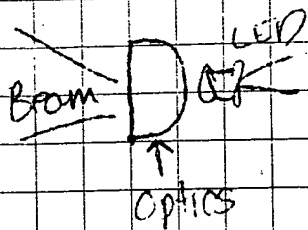
*



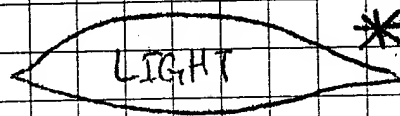
Back View



Side View



BEAM SHAPE:

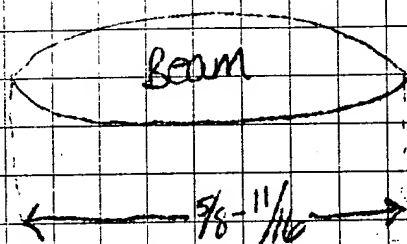


* Can increase height & width by position of LED

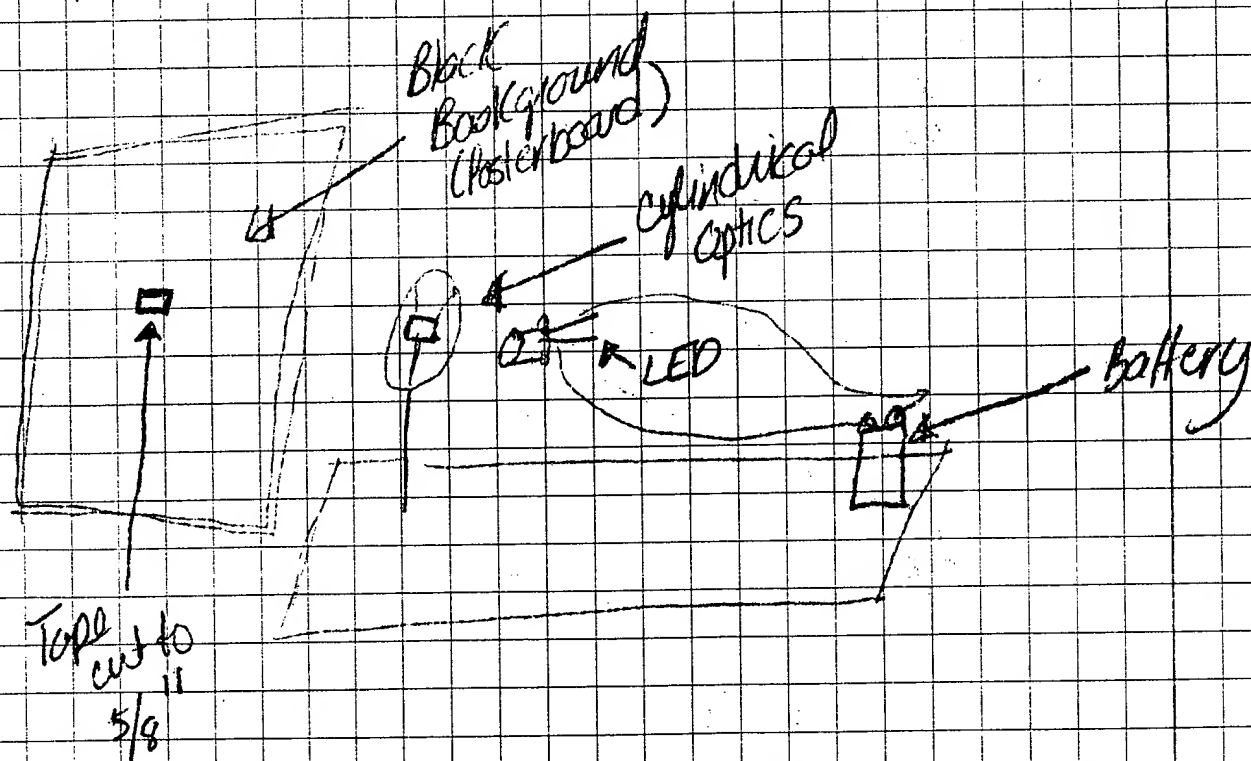
February 6, 2001

Size (length of) flow-tube illumination

$5/8'' - 1/16''$



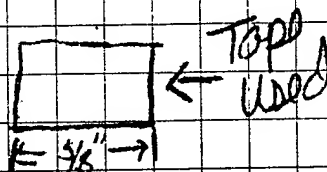
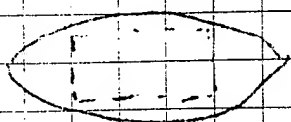
Experiment to find beam LED distance from flow tube



- Put LED butted up against optics.
- Shine LED beam w/ optics on tape
- Move posterboard forward/backward until beam is just the size of the tape.

Configuration #1

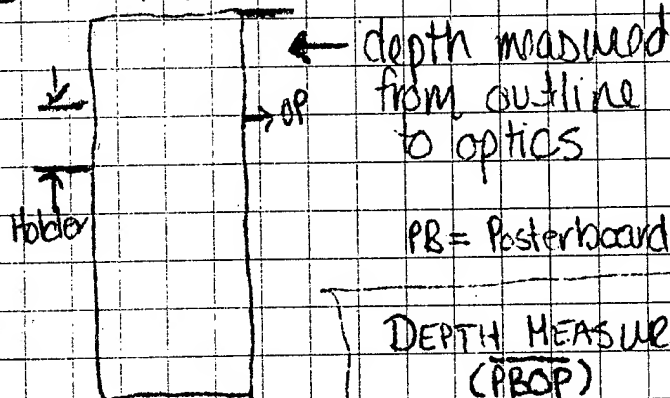
* With this experiment, posterboard also butted up against optics holder provide ample light!



* Make outline on posterboard of beam shape & take measurements

①

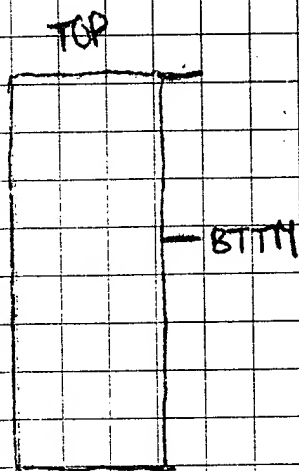
PB



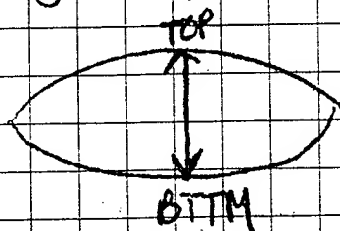
PB = Posterboard Outline

DEPTH MEASURED = $9/16$ "
(PBOP)

②

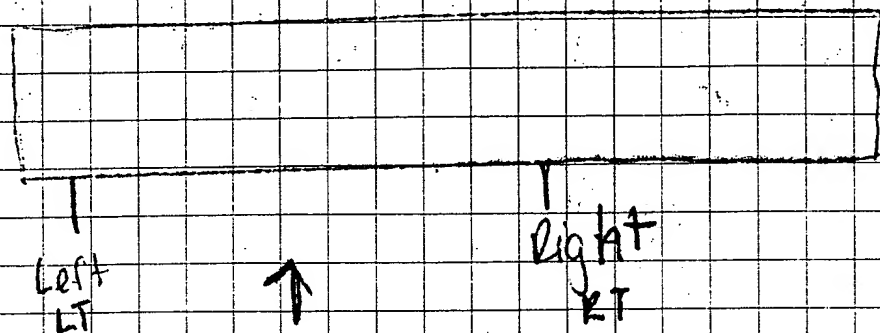


← height of beam measured
② $9/16$ " depth
(highest portion → middle)

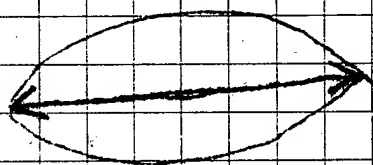


HEIGHT MEASURED = $14/16$ " = $7/8$ "
(TOP BTM)

3

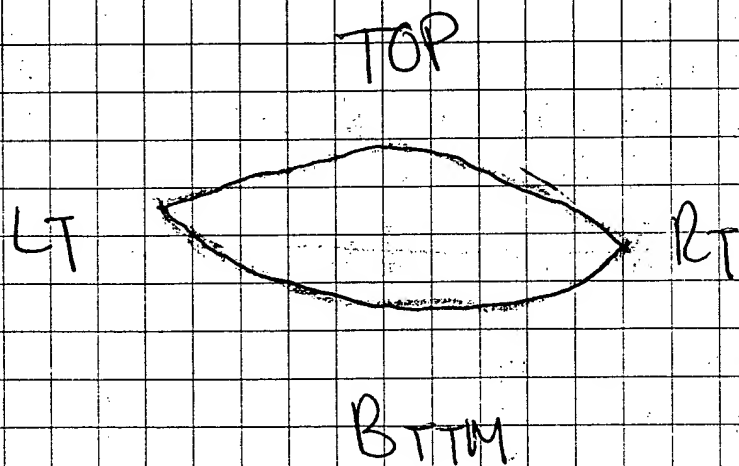


Length of beam @ depth of ~~1/16~~ $9/16$ "



LENGTH MEASURED = $2\frac{1}{2}$ "
(LT-RT)

④ Traced Version of beam outline:



February 15, 2001

* Need steady rectangular beam of

9 Volt battery $\rightarrow V = IR$

$$I = \frac{V}{R} = \frac{9V}{1000\Omega} = 9mA$$

12V battery $\rightarrow I = \frac{12V}{1000\Omega} = 12mA$

Should only use up to 20V power source

EXHIBIT N

Date: Wed, 21 Feb 2001 10:50:14 -0700
From: "David F. Langiulli" <DLang@uwyo.edu>
Subject: US Patent Filing for UW 00-004 Entitled LED Flow Cytometer Illumination (Johnson)
To: "Jennifer Bales (E-mail)" <bales@mbj-law.com>
Cc: "Paul E. Johnson" <PJohnson@uwyo.edu>,
"William A. Gern" <WillGer@uwyo.edu>,
"Rodney P. Lang" <RLang@uwyo.edu>, Roger Wilmot <RWilmot@uwyo.edu>
Importance: high
X-Priority: 1

Jennifer:

Good speaking with you this morning. Please see Letter of Engagement below. As we discussed, you will review the initial application filed by Winslow to see if there was any mention of LED's in order that we might be able to take advantage of that priority date.

Please call me if you have any questions.

DL

David Langiulli, Director
University of Wyoming Research Products Center
P.O. Box 3672, Laramie, WY 82071
Tel: 307-766-2509 Fax: 307-766-2530 Cell: 307-760-1962
Email: DLang@uwyo.edu
Web Site: <http://www.uwyo.edu/rpc>
Helping Wyoming Inventors/Entrepreneurs Identify, Protect & Commercialize Intellectual Property

Letter of Engagement for the Patent Prosecution of UW/Softray Invention
Entitled:

"LED Flow Cytometer Illumination" (Paul Johnson)
UW Technology ID No. : 00-004

Dear Jennifer:

This is to confirm that the University Of Wyoming (UW), with authority from the Office of University Counsel, has retained you to file a United States Patent Application on the invention of Professor Paul Johnson entitled "LED Flow Cytometer Illumination". This application should be filed no later than March 2nd, 2001. Please let us know immediately if you will not be able to meet this deadline.

As per our discussion, it is our expectation that total billings for all costs associated with preparing and filing the application, drawings, oath of declaration, and an assignment (jointly between UW and Softray), including attorney time, fees, and any out of pocket expenses, will not exceed \$4,500. Please inform us immediately if you anticipate expenses to exceed this amount. All invoices should be itemized, and should be forwarded to me at the Wyoming Research Products Center (RPC). In order to properly access the scope of the work accomplished and keep our files current, it is essential that all related documents be in UW's possession. Any documents

not previously forwarded must be attached to the invoice. Attorney invoices will not be considered for payment until our office is in receipt of the necessary papers. Following approval of the expenditures, RPC will forward the invoice to the appropriate internal or external party(s) for payment.

Please note that in the absence of a license or obligation to license inventions to large entity(s), UW is eligible to claim small entity status and thereby pay reduced patent office fees. This is the case for this invention. Please be sure that UW pays fees at the reduced rate.

As you are aware, RPC has overall responsibility for the management and licensing of the UW patent portfolio. We therefore ask that you provide this office with (1) a copy of the application and filing receipt, (2) an original assignment of the invention jointly to UW and Softray at the time of filing the patent application, (3) copies of all correspondence with the inventors, (4) copies of all correspondence with the Patent Office, including office actions, responses and amendments, and (5) copies of other documents that may reasonably be of value. It is our intention that the UW file be as complete as that which you maintain in your own office. It is only with such complete information that we can professionally advise our faculty inventors and knowledgeably discuss the invention with potential licensees. Please also copy the inventors on any correspondence relative to them i.e., office actions, responses and amendments.

UW has assigned Technology ID Number 00-004 to this technology. Please be certain to include this number prominently on all correspondence and copies of documents forwarded to RPC regarding this case. Failure to include this ID number on relevant documents, particularly invoices, may substantially delay payment.

Although this office is responsible for overseeing the patenting process, it is not our intention to intercede in or interfere with the open communication between UW inventors and their patent counsel. Please feel free to communicate directly with the inventors regarding all technical matters of the application as you feel necessary for the proper and expedient prosecution of the case. Of course, if you should experience any difficulty in obtaining necessary information, documentation, or other assistance from the inventors, please let us know so that we can actively seek to alleviate such difficulties. Furthermore, all formal matters and decisions regarding conversion to PCT, continuation or abandonment, division, appeal, and costs should be brought to the attention of RPC rather than to the inventors.

You were selected by UW because of your unique technical background and level of skill. Therefore, you will personally attend to the drafting and prosecution of all technical aspects of the patent application(s) contemplated, with staff support on formal and administrative aspects. Any change in this commitment must be approved by UW.

Upon receipt of an official office action, please forward it to RPC, with a copy to the inventors for their comments. If you do not receive notice to the contrary from RPC within 30 days, you may assume that UW wishes you to respond to the office action. It is expected that your billings for preparing and filing a response will be in addition to that billed for initial filing, and that billing for your services will be at the same hourly rate.

We look forward to working with you on this project.

Sincerely,

David Langiulli, Director
Wyoming Research Products Center

EXHIBIT M

Status: U
Date: Fri, 01 Nov 2002 15:12:13 -0700
From: "Paul E. Johnson" <PJohnson@uwyo.edu>
Subject: FW: Patents
To: bales@mbj-law.com
Thread-Topic: Patents
Thread-Index: Ab/8DrlddLH4PWezEdSu6ABAM9pw7gAsUwFAAAJ5+4CFCILVoAAAGhYg
X-OriginalArrivalTime: 01 Nov 2002 22:12:13.0153 (UTC)
FILETIME=[BAD7B510:01C281F3]

-----Original Message-----

From: Paul E. Johnson
Sent: Friday, November 01, 2002 3:09 PM
To: Paul E. Johnson
Subject: FW: Patents

-----Original Message-----

From: David F. Langiulli
Sent: Wednesday, August 02, 2000 3:32 PM
To: Paul E. Johnson
Subject: RE: Patents

No problem. As I have said, I know of a couple of good attorneys that we can use.

DL

David Langiulli, Director
University of Wyoming Research Products Center
P.O. Box 3672, Laramie, WY 82071
Tel: 307-766-2509 Fax: 307-766-2530 Cell: 307-760-1962
Email: Dlang@uwyo.edu
Web Site: <http://www.uwyo.edu/rpc>

> -----Original Message-----

> **From:** Paul E. Johnson
> **Sent:** Wednesday, August 02, 2000 2:28 PM
> **To:** David F. Langiulli
> **Subject:** RE: Patents

>
> David,
>
> Thanks for being patient. I talked to Winslow about the
> possibility of dropping the LED patent. He will tell me in
> two weeks whether or not he has too much on his plate. Right
> now I am inclined to have another party work on that patent.
> Let's try to make a plan of action shortly, by the end of
> August in any case.
>
> Paul
>

> -----Original Message-----

> **From:** David F. Langiulli
> **Sent:** Tuesday, August 01, 2000 5:12 PM
> **To:** Paul E. Johnson
> **Subject:** Patents
>
> In addition to talking about our strategy and tactics for the
> Dakota meeting, we should talk about the status of the
> patents with Winslow. I recently got a good referral of a EE
> patent attorney.
>
> DL
>
>
> _____
> **David Langiulli, Director**
> **University of Wyoming Research Products Center**
> **P.O. Box 3672, Laramie, WY 82071**
> **Tel: 307-766-2509 Fax: 307-766-2530 Cell: 307-760-1962**
> **Email: Dlang@uwyo.edu**
> **Web Site: <http://www.uwyo.edu/rpc>**
>
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